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VACCINATION IN INDIVIDUALS/PATIENTS AND POPULATIONS AT RISK

Lise BOEY

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List of abbreviations

9vHPV	Nine-valent HPV
ACIP	United States Centers for Disease Control and Prevention Advisory Committee on Immunization Practices
AE	Adverse event
ANSS	All type-specific naïve subjects with serology
ART	Antiretroviral therapy
CAP	Community-acquired pneumonia
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CIN1	Cervical intraepithelial neoplasia grade 1
CIN2	Cervical intraepithelial neoplasia grade 2
CIN3	Cervical intraepithelial neoplasia grade 3
CKD	Chronic kidney disease
cLIA	Competitive Luminex® immunoassay
COPD	Chronic obstructive pulmonary disease
CRPS	Chronic regional pain syndrome
DM	Diabetes mellitus
DTP	Diphtheria-tetanus-pertussis
ECDC	European Centers for Disease Prevention and Control and Prevention
ELISA	Enzyme-Linked Immuno Sorbent Assay
ESEN	European Sero-Epidemiology Network
FHA	Filamentous hemagglutinin
GMT	Geometric mean titer
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GVAP	Global vaccine action plan
HAI	Hemagglutination inhibition
HBV	Hepatitis B virus
HCI	Healthcare institution
HCW	Healthcare worker
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HSIL	High-grade squamous intraepithelial lesions
IgG	Immunoglobulin G
ILI	Influenza-like illness
IPD	Invasive pneumococcal disease
JCVI	The Joint Committee on Vaccination and Immunisation
KDIGO	Kidney Disease Improving Global Outcomes
LLOQ	Lower limit of quantification
LSIL	Low-grade squamous intraepithelial lesions

LTCF	Long-term care facility
MMR	Measles-mumps-rubella
mMU	Milli Merck unit
MSD	Merck Sharp & Dohme
MSM	Men who have sex with men
NITAG	National Immunization Technical Advisory Group
NYHA	New York Heart Classification
OR	Odds ratio
PCV13	13-valent pneumococcal conjugate vaccine
PID	Primary immunodeficiency
PLWH	People living with HIV
POTS	Postural orthostatic tachycardia syndrome
PPSV23	23-valent pneumococcal polysaccharide vaccine
PRN	Pertactin
PT	Pertussis toxin
qHPV	Quadrivalent HPV
RCT	Randomized-controlled trial
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts
SHC	Superior Health Council (Belgian national immunization technical advisory group)
SOT	Solid organ transplantation
Tdap	Tetanus-diphtheria-acellular pertussis
TGF-β	Transforming growth factor-β
TIP	Tailoring Immunization Programs
TNF-α	Tumor necrosis factor-α
VLP	Virus-like particle
WHO	World Health Organization

CHAPTER 1: Introduction

1. Vaccine preventable diseases: a continuing threat

Vaccination prepares the immune system for an encounter with infectious bacterial or viral pathogens and hence acts to prevent disease. Except for the availability of clean water and sanitation, the routine practice of vaccination is the best public health intervention to prevent and control infectious diseases. It is estimated that vaccination has avoided more than 100 million cases of smallpox, measles, polio, rubella, mumps, hepatitis A, diphtheria, and pertussis. Annually, vaccines prevent 2.5 million children's deaths. Vaccination led to the eradication of smallpox in 1979, which has already prevented 350 million smallpox cases and 40 million deaths [1]. In addition, vaccination has reduced poliomyelitis cases with 99.9% and has eradicated wild-type polio type 2 and 3. Polio wild type 1 is currently only endemic in Pakistan and Afghanistan (situation in September 2020) [2]. Vaccination has also led to control and elimination of vaccine-preventable diseases in many countries and substantially reduced the burden of several infectious diseases, such as influenza, measles, mumps, rubella, diphtheria, tetanus and pertussis [3]. Moreover, vaccines have the potential to prevent cancers induced by infectious pathogens, such as human papilloma virus (HPV) and hepatitis B virus (HBV).

It is necessary to reach and maintain high vaccination coverage for vaccination programs to be successful. Unfortunately, high coverages are not always reached for various multifactorial and complex reasons, including a lack of affordability or access and public distrust. As illustrated in figure 1, the decreasing occurrence of a vaccine-preventable disease due to vaccination coincide with the increased occurrence of side effects (because a vaccine is used more). This leads to the perception that side effects are more common than the vaccine-preventable disease and this can results in a rise in vaccine decliners and disease outbreaks [4].

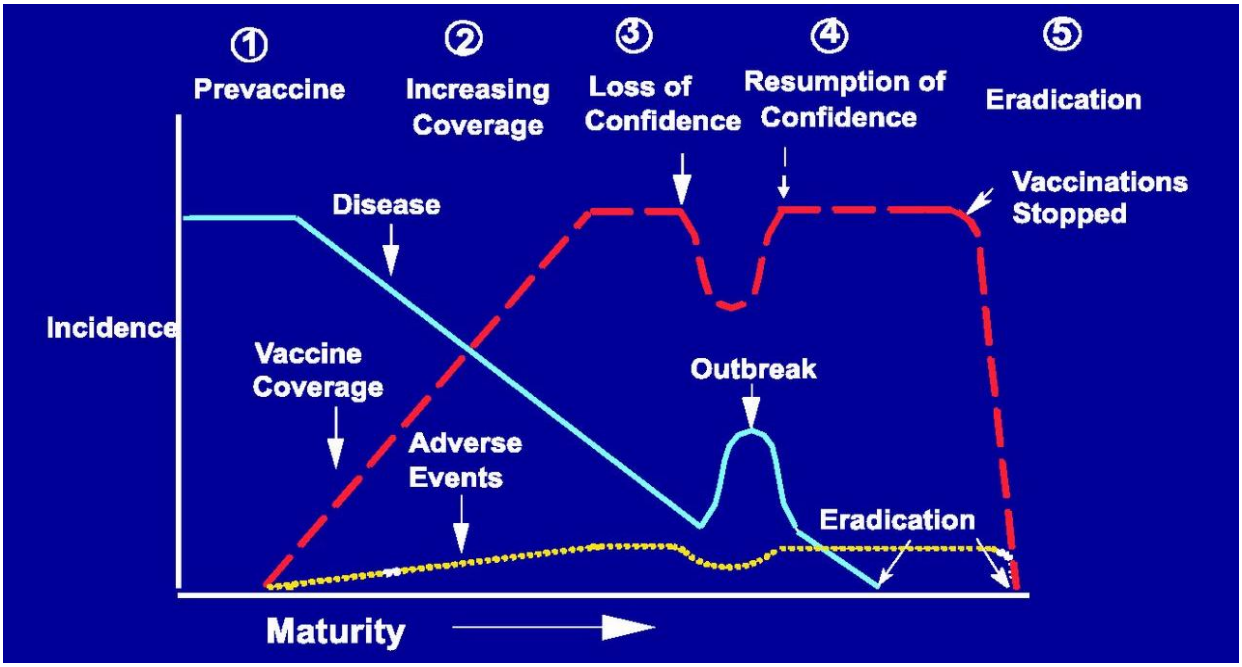

Figure 1: **Evolution of a vaccine program.** Reproduced from Chen RT, Orenstein WA. Epidemiologic methods in immunization programs. Epidemiol Rev. 1996;18(2):102. Copyright © 1996 by the Oxford University Press [4].

The World Health Organization (WHO) defined vaccine hesitancy, which is the delay in acceptance or refusal of vaccination despite its availability, as one of the ten global health threats in 2019 [5]. Suboptimal vaccination is a continuing threat to public health. On the one hand, unvaccinated persons have an increased risk of contracting infectious diseases. For example, studies have shown that unvaccinated children were 22-35 times more likely to acquire measles and 6 times more likely to acquire pertussis than vaccinated children [6,7]. On the other hand, unvaccinated individuals can also pass on infectious diseases to individuals who cannot be vaccinated or in whom vaccination does not produce a sufficient immune response, such as immunocompromised patients.

Due to suboptimal uptake or delay in vaccination, primary vaccine failure, secondary vaccine failure or waning immunity, 1.5 million children still die of vaccine-preventable diseases every year [8]. This comes down to one vaccine-preventable death every 20 seconds. Moreover, many people are still living with cancers or disabling diseases caused by pathogens against which they could have been vaccinated [8]. Some vaccine-preventable diseases, such as measles, mumps and pertussis, have also resurged over the past decades. In Europe, the number of measles cases has been increasing since 2016. For example, there were as much as 17 822 cases in 2018 and still 13 200 in 2019, and 37 deaths in 2018 and 10 in 2019 the EU/EEA/UK member states [9,10]. In Belgium, in particular, there were 367 cases in 2017, 118 in 2018 and 496 in 2019, whereas there were only about 60 annual measles cases between 2013 and 2016 [9]. Worldwide there were 10 million infections and 142 000 deaths related to measles in 2018 [11]. This is a marked increase since 2017, in which there were 7 585 900 cases and 124 000 deaths [11]. Similarly, for mumps, increased number of cases have been occurring since 2011, particularly among students. In 2011 there was an outbreak in the Belgian student population that originated from Belgium's neighboring country, the Netherlands, and which further spread to the general population [12]. In 2016, 14 795 cases of mumps were reported in Europe [13]. Cases have occurred in vaccinated individuals due to waning immunity in the absence of natural boosting. Pertussis cases have been slightly increasing since 1998 and more pronouncedly since 2012. The WHO reported 151 074 cases in 2018 and estimates 89 000 annual deaths [14]. In Europe, there were 42 242 cases of pertussis in 2017 [15]. In Flanders, pertussis cases have been increasing since 2011 [16]. A seroprevalence study in the Netherlands showed an increase of high anti-PT titers over the recent years from 4% in 1995 to 9% in 2006, which indicates increased circulation of pertussis [17]. This rise has been related to a switch from whole-cell pertussis to acellular pertussis vaccines. Although the majority of cases occurs in infants and adolescents, adult cases occur at highly underestimated rates as the typical whooping cough does not display. It is even suggested that 2% of the population gets infected annually with pertussis and that 13-20% of the cases of long-lasting cough are caused by pertussis [18].

1.1. Vaccine-preventable diseases: a focus on Human Papilloma Virus (HPV)

Human papilloma virus

Human papilloma virus (HPV) is a small circular non-enveloped double-stranded DNA virus, member of Papillomaviridae family. The DNA has 7900 base pairs and is enclosed in a capsid shell with major (L1) and minor (L2) capsid proteins. HPV DNA encodes for oncoproteins E6 and E7, which inhibit p53 and pRB tumor suppressors, and hence make HPV oncogenic. There are over 150 types of HPV defined, of which 12 types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are known to be high-risk types due to their invasive cancerogenic characteristics. Low-risk HPV-types, such as HPV 6 and 11, generally cause mild disease including anogenital warts and respiratory papillomatosis [19].

Burden of HPV

HPV is the most common sexually transmitted disease and causes about 5% of all cancers worldwide. It is a necessary cause of cervical malignancies and contributes to 90% of anal cancers, 60-90% of vaginal cancers, 40-50% of vulvar and penile cancers and, to a lesser extent, oropharyngeal and mouth cancers [20]. Cervical cancer is the fourth most common cancer in women worldwide. In 2018, there were 570 000 new cases of cervical cancer and 311 000 lethal cases [21]. There is also a growing recognition of HPV disease in men, which contributes to about 1% of all cancers in Europe. Globally, there were another 124 000 cases of HPV-related cancers reported in 2018 (in addition to cervical cancer cases), of which about 70 000 occurred in men [22]. Overall, up to 80% of women get infected with HPV during their lives, with the highest prevalence occurring 3 to 4 years after sexual onset. For men the risk of infection starts shortly after sexual debut and remains high throughout their life, with an estimated lifetime probability of over 90% for those with at least one opposite sex partner [23].

HPV life cycle

HPV infects either mucosal or cutaneous epithelia, but usually gets cleared within 12-24 months [22]. In the limited subset in which it persists longer, HPV infection might evolve to low-grade dysplasia and eventually high-grade dysplasia and invasive cancer (figure 2). On average, it takes about 5 to 10 years to go from infection to high-grade dysplasia, another 5-20 years to go to invasive cancer and 1 to 5 more years to cause death [24,25]. Risk factors for HPV infection are sexual behavior, early age of sexual onset, having new sex partners and number of life time sexual partners. Risk factors for persistent infection are smoking and oral contraceptives [19].

The life cycle of HPV is complex and especially studied for cervical HPV as it accounts for about 85% of HPV disease. HPV enters the basal cell layers of the epithelium through microtrauma and can infect basal keratinocytes (figure 2) [19]. In a first phase the virus replicates slowly and viral genome is kept as a low copy number episome (i.e. 50-100 copies per cell) in the basal cells [26]. During the second phase, which takes place in the suprabasal cells, the viral genome amplifies and capsid proteins are produced. Eventually, virions will be assembled in the upper epidermis, viruses will be shed and oncoproteins will be produced. This process includes different challenges for the host's immune system. Firstly, HPV uses the differentiation process of keratinocytes which are shielded from circulating immune cells. Secondly, virion shedding occurs at the upper epidermis and hence does not cause viremia. Thirdly, HPV does not cause cell lysis as keratinocytes die as part of their own differentiation process. Moreover, HPV downregulates multiple immunological pathways, inhibits recruitment of dendritic cells

and inhibits Langerhans cell activation [27]. Although the virus can evade the host's immune system for a period of time this way, the virus gets cleared in most cases. Virus clearance is highly dependent on cellular immunity and requires CD4+ T helper cell response and infiltration of CD8+ T killer cells cross-primed by antigen-presenting dendritic cells [27]. During clearance, infected keratinocytes produce transforming growth factor-β (TGF- β) and tumor necrosis factor-α (TNF-α), which are also produced by recruited immune cells [28]. Both cytokines inhibit the cell-growth and suppress the expression of E6 and E7 oncoproteins.

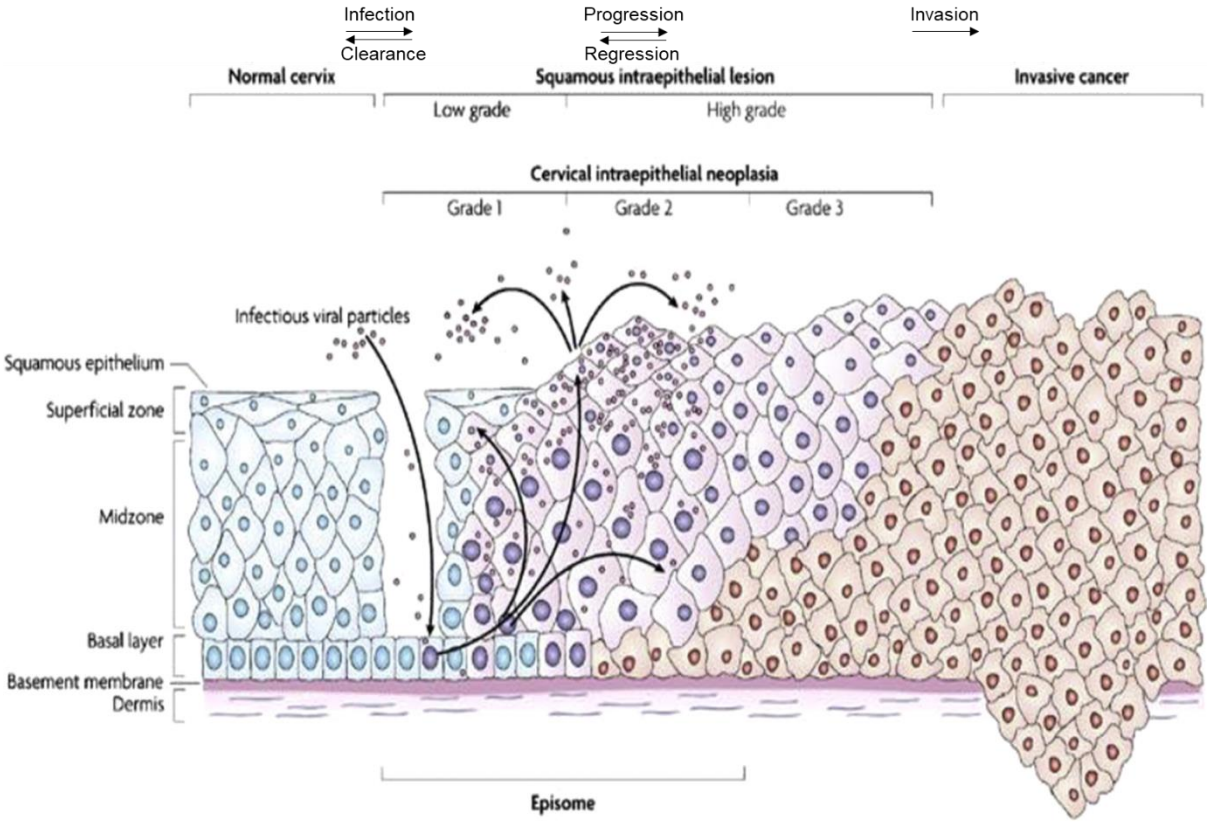

Figure 2: HPV life cycle. Schematic representation of HPV life cycle in the cervix going from infection in the cervical mucosa to invasive cancer**.** Figure adapted from De Sanjosé et al. [19]

HPV Vaccines

So far, three preventive vaccines have been authorized against HPV: the bivalent vaccine (Cervarix) against HPV type 16 and 18, the quadrivalent HPV (qHPV) vaccine (Gardasil®) against HPV types 6/11/16/18 and the nine-valent HPV (9vHPV) vaccine (Gardasil®9) against HPV types 6/11/16/18/31/33/45/52/58. The nine-valent vaccine contains 5 additional virus-like particles (VLPs) of oncogenic HPV types compared to the qHPV vaccine. Consequently, it has an additional preventive benefit, as it increases the coverage of cervical cancer-causing HPV types from 70% to 90% [29]. The qHPV and bivalent HPV vaccine have been licensed in more than 100 countries since 2006 and 2007, respectively. The 9vHPV vaccine was licensed in the US in 2014 and in Europe in 2015. Gardasil®9 is currently approved for use in persons (boys and girls) as of nine years of age (Belgian summary of product characteristics).

Safety, immunogenicity, efficacy and effectiveness of HPV vaccines in the general population

All HPV vaccines are safe and well tolerated. Common side effects include injection-site reactions such as pain, swelling and erythema, low grade fever and headache. These side effects are transient and mostly mild to moderate in intensity [30]. Injection-side reactions occur somewhat more frequently with Gardasil-9 and Cervarix compared to Gardasil, but the safety profile is favorable for all three vaccines. During post-licensure surveillance, several diseases such as auto-immune diseases and acute disseminated encephalomyelitis have been reported. However, for none of these diseases a causal relationship with HPV vaccination could be established [31,32]. Despite this, rumors about adverse events (AEs) and media attention to a series a case stories have posed a threat to vaccination coverage in countries like Denmark, Japan and Ireland [33–35]. Moreover, it preceded an increase in reported AEs, which raised concerns about a potential relationship between HPV vaccination and CRPS (chronic regional pain syndrome) and POTS (postural orthostatic tachycardia syndrome). Following this, the European medicine agency's Pharmacovigilance Risk Assessment Committee performed a review on HPV vaccine safety on request of Denmark and concluded that evidence does not support a causal relationship between HPV vaccination and POTS or CRPS [36]. The Irish government rapidly replied to the dissemination of anecdotical stories by organizing a vaccination campaign. This way they restored their vaccination coverage. Japan, on the other hand, suspended the recommendation to vaccinate girls against HPV in 2013, two months after it had been implemented in the national immunization program. In the latter country, vaccination coverage dropped from over 70% to less than 1% [35].

With regards to immunogenicity, seroconversion rates of virtually 100% have been found, regardless of sex and age of the investigated populations. For the 9vHPV vaccine, immunogenicity has been tested in males and females between 9-26 years of age [37–40] . GMTs are negatively correlated with age and body mass index, lower in women compared to heterosexual men and in MSM compared to heterosexual men [40].

Vaccine efficacy is proven for the bivalent vaccine (PATRICIA, Costa Rica Vaccine and VIVIANE trials) and qHPV vaccine (FUTURE trials). Particularly, the **bivalent vaccine** showed 100% efficacy in preventing cervical intraepithelial neoplasia grade 3 (CIN3) caused by HPV16/18 and 88% efficacy in preventing CIN3, irrespective of causing HPV-type, in young women (15-25 years) [41,42]. For older women (25-45 years), efficacy of 81% against persistent HPV infection and cervical intraepithelial neoplasia grade 1 (CIN1) was shown [43]. Concerning the **qHPV vaccine**, efficacy of >95% in preventing cervical, vulvar and vaginal intraepithelial neoplasia and anogenital warts caused by HPV6/11/16/18 was initially demonstrated in young women (16-26 years) (FUTURE I/II trials) [44,45]. Subsequently, the qHPV vaccine showed to be about 90% efficacious against persistent HPV infection, cervical intraepithelial neoplasia and anogenital warts in older women (24-45 years) [46,47]. Moreover, the qHPV vaccine proved to be preventive for HPV infection, anogenital warts and anal dysplasia in men (16-26 years) [48,49]. The **9vHPV vaccine** is effective in preventing HPV infection in boys and girls (9-15 years of age) and men and women (16-26 years). In particular, a study in women (16-26 years) showed that the vaccine was 96% effective in preventing high-grade cervical, vulvar or vaginal intraepithelial neoplasia caused by HPV31/33/45/52/58 and that antibody titers against HPV6/11/16/18

were none-inferior to those induced by the qHPV vaccine [39]. In addition, 9vHPV vaccine-induced antibody titers in girls and boys (9-15 years), and in men (16-26 years) that were non-inferior to those of young women (16-26 years) [37,40]. This supports bridging of the efficacy results of the young women (16-26 years) to boys and girls (9-15 years) and men (16-26 years). 9vHPV immunogenicity data in people over the age of 26 are not yet available.

A study on long-term protection showed that the **bivalent vaccine** has 100% vaccine efficacy against cervical intraepithelial neoplasia grade 2 (CIN2) for at least 11 years after initial administration [43]. In terms of the **qHPV vaccine**, long term effectiveness in prevention against precancers caused by HPV16/18 lasts for at least 10 years with a trend for continued protection through 12 years of follow-up [50]. An interim analysis of a long-term follow-up of an efficacy trial of the **9vHPV** vaccine showed that the vaccine provides protection against HPV16/18/31/33/45/52/58-related (pre)cancers for at least 6 years with a trend towards continued effectiveness for up to 8 years [51].

A recent meta-analysis that assessed real-world evidence of the impact of HPV vaccination in 14 high-income countries, found a significant reduction in infections with HPV16/18, incidence of anogenital warts and CIN2 among women and girls 9 years after the introduction of HPV vaccination [52]. Moreover, cross-protection was observed since infection with HPV31/33/45 decreased among women ≤ 20 years. As it generally takes about 20 years to go from infection to invasive cancer, studies have not yet been able to demonstrate protection against invasive cancer. However, evidence for reduction of the cause (HPV infection) and CIN2, the nearest proxy of cervical cancer, indicates that HPV vaccination has the potential to reduce cervical cancer [52]. Besides, a Scottish real-world evidence study showed nearly 90% reduction in CIN3 in individuals vaccinated with the bivalent vaccine at 12-13 years of age [53]. Moreover, a Finnish cancer-register study found an incidence of invasive cancer of 6.4% in unvaccinated young women and no cases in same-aged unvaccinated women [50]. This study reported a vaccine effectiveness estimation of 100%, but with a broad confidence interval due to the small endpoint frequencies. Nevertheless, this is a first indication of effectiveness in preventing HPV-related invasive cancer.

Dynamics of HPV programs

HPV vaccination was initially implemented in many countries as a 3-dose regimen for young girls. Following new immunogenicity data and the recommendation of the WHO in 2014, 2 dose regimens have been implemented for adolescents under the age of 15 in many countries. Currently, some studies are suggesting that even 1-dose schedules would be sufficient. Over the years the HPV burden in men has been increasingly recognized. The risk of acquiring HPV does not change throughout their life, they have higher HPV prevalence, prevalence of oropharyngeal cancer is 4-5 times higher and they have a higher case-fatality rate as no routine screening is performed [54]. For these reasons, many countries are implementing a gender-neutral vaccination program, in which both girls and boys are being vaccinated. Such universal programs have the advantage of providing equality in use of health service and reduce the transmission to women. In order to increase the incidence of HPV disease more rapidly, experts have suggested to combine HPV screening in women with preventive vaccination, a project called HPV-FASTER [55]. Since it is known that HPV vaccination does not reduce progression to

precancerous stages in women who are HPV infected at the time of vaccination, the ideal time to get vaccinated is before sexual onset and before HPV acquisition. However, adults can still gain protection against HPV-types they have not yet acquired.

1.2. Vaccine-preventable diseases: a focus on seasonal influenza

Influenza viruses

Influenza viruses A, B and C are three genera of the *Orthomyxoviridae* family that are characterized by segmented single-stranded, negative RNA [50]. Influenza D also exists, but no human cases have been reported. Influenza A and B have 8 segments of negative RNA and encode for at least 10 viral proteins. Influenza C, on the other hand, encodes for 7 segments of negative RNA and encodes for 9 viral proteins. Influenza A, B and C are differentiated depending on the antigenic characteristics of the viral proteins nucleoprotein (NP) and matrix protein 1 (M1). Influenza A and B are currently circulating and cause annual epidemics, while influenza C only causes sporadic cases. Influenza A causes the highest mortality and morbidity and is the only strain known for having caused pandemics. Human influenza A viruses originate from water birds and are classified according to the type of surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). These are surface glycoproteins that are important for virus entry to the host cell and viral shedding, respectively. Combinations between the 18 types of hemagglutinin and 11 types of neuraminidase are possible. Currently, influenza A subtypes H1N1 and H3N2 are circulating in humans. Influenza B viruses cause a less severe course of diseases. They have evolved over time, presumably from a temporarily circulating human influenza A virus. Influenza B only host humans and is named after the place and year of its first occurrence. Currently, there are two influenza B lineages, B/Victoria and B /Yamagata, circulating in humans.

Seasonal influenza

Seasonal influenza is a highly contagious acute viral infection caused by influenza viruses. It spreads easily through droplet aerosols produced by the coughing or sneezing of infected people. Influenza primarily infects and replicates in columnar epithelial cells of the respiratory tract, but can replicate throughout the complete respiratory tract [56]. After deposition on epithelial cells, the viral surface glycoprotein HA attaches to a cellular receptor for virus entry via endocytosis. Subsequently, infected host cells replicate the viral RNA and assemble and shed new virions. Due to influenza infection, epithelial host cells become vacuolated, lose cilia and become necrotic [56]. It can take up to 3 to 4 weeks until epithelium is restored, which can be accompanied by continued respiratory complaints. Virus can be isolated from an infected person from the day before symptom onset until 3 to 5 days after symptoms have ceased. Virus shedding is the highest 1 to 3 days after symptom onset, which is the period during which the virus is the most transmissible. Children often shed virus for a longer period of time and at a higher concentration, and hence have a substantial contribution to disease transmission.

The incubation period ranges from 1 to 4 days, and influenza symptoms range from mild to very serious or even death. The main symptoms are fever, muscle and joint pain, cough, sore throat, runny nose and severe malaise [57]. It is the combination of respiratory complaints with one of the other complaints that distinguishes influenza from other respiratory viruses. The most common influenza complications are bronchitis and pneumonia, and otitis media in children. However, other complications, such as myositis,

myocarditis, pericarditis, encephalitis, multi-organ failure, toxic shock syndrome and Reye's syndrome (in children undergoing long-term aspirin therapy) may also occur. Complications can be caused directly by the influenza virus or by bacterial of fungal surinfection with for example *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Aspergillus* [58,59]. Complications mainly occur in elderly, and more than 95% of lethal cases occur in persons above the age of 65 [60]. Infants and persons with co-morbidity are also more likely to develop a serious course. Without complications, complaints generally last for about three to seven days, but cough and malaise can last for more than two weeks. With the occurrence of the new SARS-CoV-2 virus, a new coronavirus that had its origin in Wuhan China, it will be more difficult to discern an infection with the influenza virus because symptoms of both viruses are overlapping.

Burden of disease

The severity of seasonal influenza epidemics varies annually and depends on the virulence of the virus strain and the match between the circulating viruses and the pre-existing immunity in the population. In general, epidemics in which the H3N2 subtype dominates cause higher hospitalization and mortality rates. Worldwide, 5-10% of the population gets infected and 3 to 5 million cases of serious illness occur annually [57]. It is estimated that 291 243 – 645 832 seasonal influenza-associated respiratory deaths (4·0–8·8 per 100 000 individuals) occur globally every year [61]. In Europe, depending on the season, there are four to fifty million symptomatic cases of influenza per year, and 15 000 to 70 000 lethal influenza-related cases [62]. In Belgium, a moderate epidemic affects about 5% of the 11 million inhabitants, and a more severe epidemic about 10% of the population. On average 1 in 1000 cases develops complications leading to hospitalization [63].

Vaccines

Inactivated influenza vaccines are globally the most commonly used influenza vaccines. They contain HA and NA of the circulating influenza viruses. Currently there are trivalent and quadrivalent vaccines. The trivalent vaccine protects against three strains of influenza (two influenza A strains and one influenza B strain) and the quadrivalent vaccine against four strains of influenza (two influenza A strains and two influenza B strains). Since 2018-2019 only quadrivalent inactivated vaccines have been available in Belgium. Since 2011, a live attenuated influenza vaccine has also been authorized in Europe for use in children and adolescents [62]. In 2020-2021 egg-based inactivated trivalent vaccines and live attenuated vaccines will contain the following influenza strains:

- A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like virus;
- A/Hong Kong/2671/2019 (H3N2)-like virus
- B/Washington/02/2019 (B/Victoria lineage)-like virus.

The quadrivalent vaccine will additionally include the B/Phuket/3073/2013-like virus strain.
Since recently, not only egg-based vaccines exist, but also cell-cultured based vaccines, which are an addition to the currently most-used egg-based influenza vaccines.

Influenza vaccine challenges

Vaccine-induced immunity against influenza is generally directed to HA and NA, which are highly immunogenic. Nevertheless, these glycoproteins are very prone to genetic modifications. Antigenic drift is caused by point mutations in HA or NA genes due to selective pressure on existing immunity and infidelity inherent to the replication of RNA genomes [64]. Such mutations at multiple antigenic sites make that the particular virus strain can no longer be successfully neutralized by host antibodies [65]. Antigenic drift occurs regularly and is the cause of the yearly new circulating variants of influenza strains. Antigenic shift, which is the exchange of a complete surface glycoprotein gene between a human influenza A virus and an animal influenza A virus, occurs much less frequently. This creates a new subtype of human influenza virus that contains a surface glycoprotein (HA or NA) derived from an animal influenza A virus [64]. Since there is no pre-existing immunity to these new variants, they have the potential to cause pandemics. The most known but also most lethal strain was the H1N1 Spanish flu strain that cost millions of lives in 1918-1920. Influenza A is prone to antigenic drift and antigenic shift [64]. Influenza B, on the contrary, only circulates in humans, which makes antigenic shift, and thus influenza B pandemics, impossible.

Due to the above-mentioned genetic modifications and limited cross-protection, annual vaccination is needed. The WHO continuously monitors the circulating strains and recommends in February which strains should be included in the vaccine for the following winter. This decision is made relatively early given that vaccine production takes about six to eight months. Consequently, mutations in the circulating strains may happen during this production period, which will render the vaccine less effective.

2. Vaccine preventable diseases: an even greater threat for at-risk groups

Certain individuals are at increased risk of developing complications and severe disease upon exposure to infectious pathogens, including those against which they can be vaccinated. This might either be due to age, pre-existing diseases or immunosuppressive treatment. These vulnerable persons are further referred to as `at-risk patients'.

Age

Ageing goes along with impaired functioning of both innate and adaptive immune functioning. This is called immunosenescence and makes the elderly more vulnerable to infectious disease. Age is a well-known risk factor for influenza complications. Whereas seasonal influenza-associated excess in mortality annually ranges from 0.1 to 6.4 per 100 000 in individuals younger than 65 years, it increases to 2.9-44.0 per 100 000 people between 65 and 74 years of age and 17.9-223.5 per 100 000 for people above the age of 75 years [61]. Moreover, the majority of people hospitalized with influenza are aged ≥65 years [66]. Also, pneumococcal disease can lead to hospitalization in as much as 73 per 100 000 among elderly and may cause death in up to 12% [67]. Another example is the incidence of zoster, which is the disease accompanying reactivation of latent varicella virus, which mounts with age. Overall, there are 3-4 cases of zoster per 1000 in Europe, but 7-8 cases per 100 000 at the age of 50 and 10 cases per 100 000 at the age of 80 [67]. In addition, diseases such as pertussis, which have especially a high burden in children, might also form a threat for elderly. The pertussis hospitalization rate has been shown to increase with age from 2.2 per 100 000 in persons aged 45-64 years to 13.5 per 100 000 in those aged over 75 years in Australia [68]. Likewise, another Australian study showed that increasing age is significantly associated with hospitalization upon pertussis infection [69].

Along with ageing, prevalence of chronic disease increases. In Flanders (North of Belgium), about 11% of the general population has a comorbidity, but prevalence mounts to 21% in persons between the age of 65 and 74 years and 42% of persons aged ≥75 years [70]. It is hence important to note that the severity of infectious diseases may as well depend on comorbidity and treatment. For example, the risk of community-acquired pneumonia (CAP), which is most frequently caused by *S. pneumoniae,* is 2- to 3-fold higher in persons aged ≥65 years with severe pulmonary disease compared to those with mild or moderate pulmonary complaints [70]. Moreover, a case-control study showed a three-fold increased probability of hospitalization due to CAP when people aged ≥65 years used corticosteroids [70].

Chronic disease

Pre-existing disease may as well contribute to severe course of disease. Intrinsic immunosuppressive characteristics of disease might either be congenital or acquired. Primary immunodeficiencies (PID) are congenital diseases that might affect any part of the immune system including the humoral or cellular immune system, complement functioning or phagocytotic functioning [71]. Acquired immunodeficiencies can, for example, be obtained through infection with the HIV virus, which severely affects the CD4+ T-cell population. Increased susceptibility to infection and a severe course of the disease depends on the degree of immunosuppression. HIV patients who take anti-retroviral therapy (ART), have an undetectable viral load and have restored CD4-cell counts, are not considered immunocompromised. On the other hand, untreated HIV-infected patients with CD4-cell counts below 200 cells/µl, patients

with advanced Hodgkin disease and patients who underwent a hematopoietic stem cell transplantation are among the most severely immunocompromised groups [72]. In some patients, immunosuppression is related to both the intrinsic immunosuppressive characteristics of the disease and the treatment. Other chronic diseases, such as diabetes, chronic kidney disease (CKD) and chronic obstructive pulmonary disease (COPD) have a more moderate effect on the immune system. Uremic state in patients with CKD, and genetic and metabolic abnormalities (high glycemic state and acidemia) and other comorbid factors (age, renal disease and cardiovascular disease) in diabetes patients are likely the main causes of disrupted innate and adaptive immune functioning [72,73]. These disruptions in immune-functioning increase the probability of developing complications. COPD is a result of long-term exposure to smoke and other environmental pollutants, which lead to a state of chronic inflammation. This leads to structural modifications such as the destruction, scaring and loss of lung tissue, but also to hyperproduction of mucus and disrupted mucociliary clearance [74]. Hence, the innate immune defense mechanisms of the lungs are disrupted, which makes the patients more susceptible to respiratory infections. Other patients with chronic diseases that are considered immunocompetent, such as patients with cardiovascular disease, might as well be at-risk for infectious diseases, as serious infections might put a strain on the cardio-vascular system.

Below, some examples of increased burden of vaccine-preventable diseases in patients with chronic diseases are shown:

- A prospective multicentric study in France found that almost 90% of patients hospitalized with laboratory-confirmed influenza had underlying conditions [66].
- HIV-infected persons in AIDS stage have a 35-fold increased probability of developing invasive pneumococcal disease (IPD) and a 10-fold increased probability to die from influenza [75]. HIV-infected persons who use anti-retroviral therapy and have an undetectable viral load have better survival rates. In addition, since HIV shares its route of transmission and hepatitis B virus (HBV), HIV-infected persons present with higher prevalence of HBV infections [76].
- Mortality in children with end-stage renal disease is most frequently caused by infections [77]. Moreover, a study found that mortality in hospitalized patients with pneumonia was higher in patients with CKD (16%) compared to other patients (8.3%) [78].
- Among diabetes patients, severe disease outcome is particularly seen in patients aged over 65 years, patients with comorbidities and poor glycemic control [70]. Patients with diabetes are at increased risk for influenza and pneumococcal disease [72].They are up to 1.4 times more likely to have CAP and 1.4 to 4.6 times more likely to develop IPD [70]. Epidemiological data also showed that diabetic patients have a twofold increased probability of acute HBV infection [79]. Moreover, having diabetes has shown to be a risk factor for progression of severe liver outcome in case of HBV infection [79].
- A study showed that pulmonary conditions, such as COPD or emphysema, are the most common underlying diseases in people hospitalized with respiratory infections [71]. This study also showed that underlying cardiac conditions were an important risk factor for influenza-related deaths [71].

- A meta-analysis showed that children with asthma are 90% more likely to develop invasive pneumococcal disease upon infection with *S. pneumoniae* [75]. Moreover, cases of *S. pneumoniae*-related pneumonia are higher among asthma patients compared to healthy controls [75].

An additional threat for patients with chronic conditions is that particular infectious diseases might aggravate existing chronic conditions. For example, infectious pathogens that cause lung disease, such as *S. pneumoniae*, *B. pertussis* or influenza might form a particular risk for patients with cystic fibrosis, asthma or chronic obstructive pulmonary disease [74,80]. In addition, influenza increases the risk for cardiac and brain infarction, which forms a risk for people with cardiovascular disease, and it might elevate blood glucose to dangerously high levels in diabetes patients [81,82].

Immunosuppressive drugs

Immunosuppressive drugs are mostly used to suppress disease-related inflammatory processes in the context of chronic obstructive pulmonary disease, immune mediated inflammatory diseases (e.g. multiple sclerosis or rheumatoid arthritis or inflammatory bowel disease), to treat cancer or to avoid graft rejection in solid organ transplant (SOT) patients and hematopoietic stem cell transplant patients. It includes glucocorticoids, antimetabolites, cytostatic drugs, monoclonal antibodies and calcineurin inhibitors. They interfere with B and/or T-cell immunity and make patients more vulnerable to complications following an infection. Below are some examples of the burden of vaccine-preventable diseases in patients who take immunosuppressive medication.

- Hospitalization for vaccine-preventable diseases occurs in more than 15% of SOT patients in the first 5 years after transplantation and at a frequency that is up to 87 times higher than in the general population [83].
- Multiple cases of acute respiratory distress syndrome and death in adult renal transplant patients upon H1N1 influenza infection have been reported [84]. Many of these cases also caused acute rejection of the transplanted kidney.
- Using corticosteroids inhalators has shown to increase the chance on CAP in COPD patients [70].

2.1. A focus on human papillomavirus in HIV and transplant patients

Studies consistently report a higher incidence of oral and anogenital HPV infections, a higher persistence of infection and a higher incidence of genital warts, anogenital squamous intra-epithelial dysplasia and HPV-related cancers in patients with HIV and SOT patients. A possible confounder is the increased attention paid to cancer screening in these populations, but the absence of a higher risk of breast or prostate cancer contradicts this argument [85].

HIV patients have a higher HPV prevalence compared to the general population [86,87]. Among HIV-positive men who have sex with men (MSM) anal HPV prevalence even ranges from 84% to 90% across all ages [88,89]. Increased prevalence can be related to higher sexual exposure or to increased susceptibility caused by HIV. It is suggested that keratinocytes that come into contact with HIV envelope glycoproteins release TGF-β and TNF-α, which downregulates tight junction proteins and E-cadherin. This way, epithelial permeability is increased and HPV virus entry into the basal cells of the epithelium

is facilitated [28]. Moreover, HPV tends to persist longer in HIV-infected people and leads more frequently to genital warts and HPV-cancers [28,90]. A meta-analysis reported standardized incidence ratios as high as 5.8 for cervical cancer, 4.4 for penis cancer, 6.5 for vaginal cancer, 28.8 for anal cancer and 2.3 for oropharyngeal cancer compared to the general population [85]. HPV-related cancers also tend to be more aggressive and to recur more frequently. Genital warts are often refractory to treatment [28,90]. HPV-related anal cancer is relatively rare in the general population, but more prevalent among HIV-positive MSM. It must be noted, however, that the incidence of HPV disease depends on the state of immunosuppression of the HIV-infected person. Antiretroviral therapy (ART), which was introduced in the late 1990s, actively reduces the HIV viral load and restores immune functioning, particularly regarding CD4-cell count. The use of ART has shown to decrease the progression of precancerous lesions to cancer, increase the regression of HPV-precancers and decrease the incidence of invasive HPV cancer [91–93]. Likewise, a high CD4 count, high nadir CD4, less time spent below <200 cells/µl and a low viral load have all been related to a better disease outcome for HIV [28,94,95]. A study even showed that HIV-infected persons with a CD4-cell count ≥500 cells/µl, do not have a worse disease outcome than the general population [96]. Incidence of anal cancer, however, has been stable or even increasing despite ART introduction. Only recently there has been some evidence of a decline [95]. Access to screening, prolonged immunosuppression and time at which ART was initiated appeared to be key factors driving this decline [97]. Evidence also exists that the nadir CD4 count and the duration of the CD4 count below 200 cells/µl are better predictors of HPV disease than the current CD4-count [95]. Since ART has not shown to impact the carriage, clearance or persistence of HPV, whether HPV infection was already present before ART initiation has limited influence [98]. HPV vaccination before HPV acquisition might help to further decrease the number of HPV-cancers in HIV patients. To this end, the 9vHPV vaccine is preferably used since high-risk HPV types other than HPV16/18 showed to have a proportionally higher contribution to HPV (pre)cancers compared to the general population [89,99].

Immunosuppressive drugs used by SOT patients are known to interfere with cellular immunity and DNA repair mechanisms. Due to the latter, these drugs have intrinsic oncogenic characteristics, leading to a two- to three-fold increased risk of any cancer compared to the general population [27]. In addition, immunosuppressive treatment counters the clearance of HPV infection and may cause reactivation of a latent HPV infection [100]. This increases the prevalence of HPV-related disease. A study reported 27.1% prevalence of HPV in the cervicovaginal mucosa, which is about three times higher than among same-aged women (45-55 years) of the general population [100]. A meta-analysis reported increased standardized incidence rates as high as 2.1 for cervical cancer, 15.8 for penis cancer, 22.8 for vaginal cancer, 4.8 for anal cancer, and 3.2 for oropharyngeal cancer compared to the general population [85]. Similarly, later studies reported a two- to six-fold increased risk of cervical cancer, a five- to 100-fold increased risk of vaginal cancer, a 10-fold increased risk of anal cancer and 5-fold increased risk of oropharyngeal cancers [28,100,101]. The incidence increased with the time since transplantation, having more than one transplantation, the use of corticosteroids, the combination of cyclosporine and azathioprine compared to tacrolimus and mycophenolate, and with being prescribed 3 or more medication classes [96,101]. HPV vaccination, preferably given before transplantation and before sexual onset, might reduce these numbers. Similarly as for HIV patients, the 9vHPV vaccine is preferably used

as there is a genotype shift of most prevalent HPV types from HPV16 and HPV18 to other high-risk types [100,102].

2.2. A focus on nosocomial seasonal influenza outbreaks

A nosocomial seasonal influenza infection is an infection that is acquired by a patient in a healthcare institution (i.e. hospital or long-term care facility (LTCFs) for elderly care) that was not present at time of admission. Nosocomial outbreaks of seasonal influenza have been reported multiple times [103–105]. During influenza seasons, infected and uninfected persons might be brought together in healthcare settings, which facilitates nosocomial transmission. For example, infected persons might be admitted and come into contact with uninfected patients (or residents) or infected persons might visit admitted patients. Moreover, healthcare workers (HCWs) are as well assumed to play a role in nosocomial transmission [103]. They cannot only acquire influenza in the community, but also in the healthcare setting, as they care for the infected residents [106]. A study showed that up to 25% of HCWs get infected with influenza annually compared to only 5% of the general population [106]. Since influenza may be transmitted before symptoms onset, HCWs can unintentionally spread influenza. Hospitalized patients and residents of LTCFs often have comorbid diseases or are ≥65 years, which brings them at-risk of a severe disease course or even death, as described earlier. Hence, influenza outbreaks in healthcare institutions are often associated with high morbidity and mortality. For example, during an influenza epidemic in a LTCF in Flanders (Belgium) in 2014, 41% of the residents showed influenza symptoms, of which 43% were confirmed by PCR. In total, 11.9% of the residents needed to be hospitalized and 4.7% of the cases were lethal [107]. Hence, influenza forms an annual challenge for hospitals and LTCFs. It leads to increased numbers of patients to be cared for, but also to higher HCW absenteeism.

3. Vaccination as a direct tool to prevent infectious diseases

Vaccines provide direct protection to a vaccinated person by mimicking a natural infection and hence preparing its immune system for an encounter with an infectious pathogen. There are two major classes of vaccines currently available. Firstly, there are live-attenuated vaccines, which include micro-organisms that are still alive and can replicate after vaccination, but which contain a weakened form of the pathogen and therefore do not cause disease. Secondly, there are the inactivated vaccines. These vaccines can contain a whole pathogen that is killed (e.g. whole cell pertussis vaccine), a limited number of antigens that are necessary to induce a protective immune response (sub-unit vaccines (e.g. inactivated influenza vaccine) or recombinant vaccines (e.g. HBV vaccine)), polysaccharides of a bacterial capsule conjugated to immunogenic protein (e.g. 13-valent pneumococcal conjugate vaccine (PCV13)) or toxoids, which are weakened forms of toxins, toxic proteins produced by bacteria such as C*lostridium tetani*. Furthermore, there are viral vectored vaccines (e.g. Ebola vaccine and dengue vaccine). Besides that, newer types of vaccines, such as RNA and DNA vaccines are under development and clinical trials for different pathogens are ongoing.

How vaccines work

When vaccines are injected, the cells of the innate immune system recognize pathogen-recognition patterns contained in the vaccine antigen or adjuvant. Subsequently, cytokines are produced and phagocytic cells, essentially dendritic cells, are attracted. Dendritic cells subsequently prepare the adaptive immune system by presenting antigen to naïve B-cells and T-cells in the lymph node, where vaccine effector mechanisms are induced [108]. Most current vaccines induce humoral immunity, which is a vaccine effector mechanism that is mediated by antibodies. Antibodies are produced by plasma B cells. The development of plasma cells occurs mainly in the draining lymph nodes of the vaccinated limb. During a complex process of maturation which takes several weeks, plasma cells that deliver high quality antibodies are generated. These final plasma cells migrate to the bone marrow of the long bones from where they produce antibodies. These antibodies help to clear pathogens by binding to the enzymatic active sites of toxins or preventing their diffusion, binding to the surface of pathogens and neutralizing viral replication (e.g., preventing viral binding and entry into cells), promoting opsonophagocytosis of extracellular bacteria (i.e., enhancing their clearance by macrophages and neutrophils) or activating the complement cascade [108]. Vaccine antigens that actively enter the cell (e.g. live vaccines, virally vectored vaccines) also induce cellular immunity or the production of CD8+ T cells after MHC class I presentation. These cells recognize and kill infected cells through direct contact or cytokine release. In order to induce and maintain humoral and cellular immunity, CD4+ T-helper cells are needed. In addition, CD4+ T-helper cells exert direct anti-microbial functions on viruses and parasites by secreting cytokines. Lastly, regulatory T-cells help to control the immune response and prevent an aberrant reaction.

All current vaccines, except for T-cell independent polysaccharide vaccines, induce immune memory [108]. Memory B-cells, developed at the same time as plasma cells, patrol throughout the body and recognize antigens from a previous encounter and subsequently differentiate into plasma B-cells that produce high amounts of high-quality antibodies. This leads to a rapid increase in high quality serum antibodies within 4-7 days. Therefore, most vaccination schedules consist of (a) priming dose(s) and a booster dose which is given 4-6 months after the priming dose to induce long-term protection. Also, antibodies can wane over time, and booster doses are sometimes given later in life to prolong protection (e.g. tetanus, diphtheria, pertussis). After boosting, antibody titers are markedly higher than after the first priming dose. Similarly, memory T-cells are quickly reactivated by an antigen and differentiate to effector T-cells. Presence of immune memory, however, does not always correlate with vaccine efficacy. In absence of circulating antibodies, efficacy appears to depend on the race between immune memory reactivation and disease pathogenesis. Regarding toxin-induced disease, it is generally assumed that protection requires antibody presence at the time of toxin exposure [108].

3.1. Correlates of protection and seroprotection

A correlate of protection is an immune function that is significantly related to protection against disease (association is not necessarily causal) [109]**.** This is often a humoral response to vaccination, although cellular immune responses may as well correlate to protection. Correlates of protection that have been defined for the standardly used vaccines are shown in table 1. Correlates of protection can be determined from pre-exposure titers, challenge studies, observations in immunocompromised patients, phase III studies, or from observations with passive immunization. In some cases, they are hard to define because immune responses that are not routinely measured might play an important role. For example, regarding pertussis, no correlate of protection has been defined. Particular antibody titers have shown to be protective in some individuals, while in others they were not. One assumes that cellular immunity plays a large role. However, some studies related the presence of antibodies against pertussis toxin (PT) and pertactin (PRN), two pertussis antigens, to protection against whooping cough. Clinical relevance of antibodies against filamentous hemagglutinin (FHA), another pertussis antigen, remains unclear. Similarly for mumps, no correlate has been defined as other immune responses such as cellular immunity and complement mediated lysis are deemed to play a role. Correlation between measles antibody titers and clinical protection was made based on blood samples collected in a school just before a measles outbreak. A correlate of protection of 120 plaque reduction neutralization titers was suggested based on 9 cases of measles who had pre-exposure plaque reduction neutralization assay titers <120 while 71 non-cases had plaque reduction neutralization assay titers >120 [110]. These titers were determined with a standard plaque reduction neutralization test, which does not entirely correspond to Enzyme-Linked Immuno Sorbent Assay (ELISA) titers with regard to antibody functionality. A more recent study, however, found cases in individuals with titers above 120 mIU/ml [111]. Moreover, other studies found waning immunity but no major outbreaks in highly immunized population [112,113]. This suggests that cellular immunity and immunological memory might as well contribute to protection, which is why a systemic review concluded that the cut-off value of 120mIU/ml should be re-evaluated [114].

Seroprotection refers to a level of immune response which is at or above a pre-defined cut-off value that corresponds with protection. In this thesis the correlate of protection cut-off values were used as stated in table 1.

Table 1: Correlates of protection

Vaccine	Measured output of immune response	Correlate of protection cut-off values
Diphtheria	Toxin Nt Ab	0.01–0.1 IU/mL
H. influenzae conjugate	ELISA Ab	0.15 ng/mL
Hepatitis A	ELISA Ab	20 mIU/mL
Hepatitis B	ELISA Ab	10 mIU/mL
Human papillomavirus	ELISA Ab	Ab, level ND
Influenza, inactivated	HI Ab	1/40 = 50% protection, 1/320 in children
	NtAb	1/40 = 50% protection
Influenza, live	HI Ab, IgA Ab, CMI	ND
Measles	ELISA Ab[a]	≥120 miU/mL
Meningococcal	Bactericidal Ab	≥1/4
Mumps	Nt Ab	ND
Pertussis	Ab to PT, Prn, Fim	ND
	Th1 T cells	ND
Pneumococcal, conjugated	ELISA Ab	0.20–0.35 µg/mL
Pneumococcal, polysaccharide	OPA Ab	ND
Polio, inactivated	Nt Ab	≥1/8
Polio, live	Nt Ab	≥1/8
Rotavirus[b]	Serum secretory IgA	ND
Rubella	ELISA Ab	≥10–15 IU/mL
Tetanus	Toxin Nt Ab	0.01–0.1 IU/mL
Varicella	GP ELISA[ ]	≥5 U/mL
Zoster	$CD4^+$ T cells	ND

[a] cellular immunity also deemed important.
Abbreviations: Ab, antibodies; CMI, cell-mediated immunity; ELISA, enzyme-linked immunosorbent assay; fim, fimbrial agglutinogens; GP, glycoprotein; HI, hemagglutination inhibition; Ig, immunoglobulin; ND, not defined; Nt, neutralization; OPA, opsonophagocytic; PRN, pertactin; PT, pertussis toxin. Table reproduced and adapted from Plotkin Vaccines, seventh edition, chapter 3; Correlates of protection [115]

3.2. Immunogenicity of vaccines in at-risk groups

Immunogenicity is the ability of an antigen to induce an immune reaction. With regard to vaccines, immunogenicity measurement mostly concerns a humoral immune response, which is the induction of antibodies against the antigen. This is mostly expressed as geometric mean titers (GMTs, or magnitude of antibody response) and seroconversion, which is having measurable antibody titers if no antibodies could be measured before vaccination or a significant increase in antibody concentration (e.g. 2-fold or 4-fold rise). Vaccine immune response can be impaired in at-risk persons due to the immunosuppressive characteristics of age, disease or treatment. The exact impact of chronic disease, age and treatment and interaction between these factors on vaccine immunology is complex and incompletely studied. Moreover, heterogeneity within a group of patients diagnosed with the same chronic disease is often high, which makes it hard to elucidate underlying mechanisms.

Elderly

Due to immunosenescence, immunogenicity deteriorates with age. A review reported significantly lower seroconversion and seroprotection against influenza vaccine antigens (H1, H3 and B antigens) with adjusted odds ratios that ranged from 0.24 to 0.59 in persons aged ≥65 years versus younger adults [116]. For this reason, some countries such as the United States have implemented increased dose and adjuvanted influenza vaccines, which all show better results [117,118]. Immunogenicity of the 23-valent pneumococcal polysaccharide vaccine (PPSV23), which is recommended for persons aged ≥65 years and individuals with chronic conditions, decreases with age, not only with regard to antibody titers but

also antibody functionality [119]. As there is no correlate of protection defined, clinical relevance of suboptimal immunogenicity is unclear. The 13-valent pneumococcal vaccine has a theoretical immunologic advantage as it stimulates T-cell immunity and induces vaccine memory [120]. Therefore, it is recommended to use both types of pneumococcal vaccines and to primarily vaccinate with the PCV13 vaccine to create immune memory and then with the PPSV23 as this vaccine covers a broader range of *S. pneumoniae* types. Better immunogenicity has been found with the PCV13 vaccine compared to the PPSV23 vaccine in adults, but superiority of one dose regimens has not been assessed in elderly [121].

Chronic disease

Primary immunodeficiencies

Many PIDs are rare, which makes that there is a lack of immunogenicity data, and available data are often insufficient and inaccurate [122]. Even for PIDs that have been known for a long time, such as IgA deficiencies, it is hard to draw meaningful conclusions due to the variability in immune functioning within a patient group with the same diagnosis.

Chronic diseases with moderate, mild or no effect on immune system

CKD patients are a heterogeneous group with regard to immune status. A study in children showed equal seroconversion rates between dialysis patients and healthy controls upon inactivated influenza vaccination. However, some other studies showed impaired immune responses. For example, studies suggested that only 69-88% of children and young adults seroconvert after vaccination with a diphtheria-tetanus-pertussis (DTP) vaccine, compared to 93-100% in healthy children [73]. Suboptimal HBV vaccine immunogenicity has also been reported. For this reason, the Belgian Superior Health Council advices to use an adjuvated HBV vaccine (Fendrix) in patients with severe chronic kidney disease. Similarly, the Advisory Committee on Immunization Practices (ACIP) in the USA recommends to use an increased dose vaccine that contains 40µg antigen instead of 20µg (Recombivax HB) or to use double doses of the standard vaccine (Engerix B) [123]. Suboptimal immunogenicity was also reported for MMR vaccination, with 70% seroconversion for measles, 50% for mumps and 80% for rubella. Moreover, studies in transplant and CKD patients showed an accelerated decline in antibody titers, particularly in diphtheria antibodies compared to tetanus antibodies [124,125].

In patients with chronic diseases that have milder or no effect on the immune system, vaccine immunogenicity is generally analogous to the one in healthy controls. For example, for influenza vaccination, seroconversion rates are generally equal to those of healthy individuals, as shown for patients with diabetes, COPD and asthma [126–128]. Furthermore, it was shown that PCV13-induced functional vaccine responses in patients with cardiovascular disease, pulmonary disease and diabetes mellitus, irrespective of prior PPSV23 administration [129]. However, comorbidities have been associated with decreased PPSV23 vaccine responsiveness [119].

Among patients with HIV, immune responses depend on treatment and CD4 cell count. A study on measles-mumps-rubella (MMR) vaccination showed reduced seroconversion in HIV-infected children compared to healthy children [130]. Another study in 626 individuals with HIV, showed that only 217

(35%) obtained protective anti-HBs titers (≥10 IU/L) [131]. However, patients who used HAART and had higher CD4 cell count were more likely to seroconvert.

Immunosuppressive treatment: solid organ transplant patients

Studies showed that SOT patients, who are under immunosuppressive treatment, less frequently mount protective titers. Decreased immunogenicity was found for influenza, diphtheria, tetanus, HBV, and pneumococcal disease [132–135]. For influenza, suboptimal immunogenicity has been related to vaccination early-post transplantation and use of mTOR inhibitors and mycophenolate. Because immunogenicity is generally suboptimal in transplant patients, it is recommended to check the vaccination status of these patients before transplantation. A study of HBV vaccination showed that protective levels were more likely reached in CKD patients who do not yet receive dialysis, compared to post-transplant patients (96.4% versus 66.7%), which supports pre-transplant vaccination [73]. Regarding the influenza vaccine, which has to be administered annually, an increased dose vaccine and simultaneous injection of two standard doses resulted in in significantly higher immunogenicity [136,137].

3.2.1. Immunogenicity of HPV vaccination in HIV and solid organ transplant patients

Several studies assessed safety and immunogenicity of the bivalent and the qHPV vaccine in HIV patients. HPV immunogenicity has shown to be related to the state of immunosuppression. Concerning the qHPV vaccine, seroconversion rates were found to be in between 95% and 100% for HPV6, HPV11/16 if the CD4 count was above 200 cells/µl. In patients with cell counts below this cut-off, seroconversion declines to about 85% for HPV6 and 90-95% for HPV11/16. Seroconversion rates are found to be slightly lower for HPV 18, and decline from between 85 and 100% to 75% when the CD4 count is below 200 cells/µl. HPV18 GMTs were found to be higher after vaccination with the bivalent vaccine compared to the qHPV vaccine. Higher GMTs have been associated with younger age, higher CD4 count and low viral load [138].

So far, there have only been four studies that reported immunogenicity of the qHPV vaccine in SOT patients. They have small sample sizes, ranging from 17 to 47 patients and show diverging results, which makes it hard to make meaningful conclusions. One study found a seroconversion rate of 100% in adolescents aged 11 to 19. It is important to note, however, that the intention to treat population only consisted of 8 patients [139]. The three other studies, two in children and one in adults, found suboptimal immunogenicity. In the first study in children, seroconversion rates ranged from 64% for HPV6 and HPV11, 74% for HPV18 to 100% for HPV16 [140]. Later than twelve months after vaccination, seroconversion dropped to 62.5% for HPV genotypes 6, 50% for HPV11, 75% for HPV16, and 50% for HPV 18, but could only be assessed in 8 patients. In the second study in children, 72.4% seroconverted for HPV6, 69.0% for HPV11, 89.7% for HPV16 and 62.1% for HPV18 [141]. In the adult study, seroconversion was lower compared to the study in children and was 63.2% for HPV6, 52.6% for HPV 11, 63.2% for HPV16 and 52.6% for HPV18 [142]. Six months after vaccination, antibodies had already declined, but the proportion of seropositive patients remained relatively stable. Studies with the bivalent and nine-valent vaccine have not yet been performed. Factors that have been associated with reduced

immunogenicity are early vaccination after transplantation, high levels of tacrolimus, and having received a lung transplantation compared to another SOT [142].

3.3. Efficacy of vaccines in at-risk groups

The WHO defines vaccine efficacy as the reduction in the probability of developing a clinical disease after vaccination, relative to the probability when unvaccinated. Efficacy is preferably defined through randomized, placebo-controlled trials (RCTs). Efficacy studies in at-risk groups frequently include relatively small sample sizes and are often of poor quality. Below, examples of vaccine efficacy in at-risk groups are given with a focus on influenza and pneumococcal vaccination.

Elderly

Whereas the seasonal influenza vaccine is about 70-90% effective in young adults if the vaccine strain matches the circulating strain, this is only about 17-53% in elderly people [131]. A meta-analysis also showed that vaccine effectiveness against laboratory-confirmed influenza in persons aged ≥65 years is only 39 to 49% [143]. Meta-analyses remain inconclusive about the efficacy of the PPSV23 vaccine in elderly (and other high-risk groups) due to heterogeneity of RCTs and lack of power [134]. However, a recent systemic review described protective vaccine efficacy and effectiveness for both the PPSV23 and the PCV13 against vaccine-type pneumonia in elderly based on observational studies [144].

Chronic disease

Primary immunodeficiencies

Similarly to vaccine immunogenicity data, efficacy data in patients with PID is lacking [122].

Other chronic diseases and patients on immunosuppressive treatment

The community-acquired Pneumonia Immunization Trial in Adults (CAPITA), which was published in March 2015 in the New England Journal of Medicine, found a vaccine efficacy against vaccine type CAP of 66.7% in healthy elderly, but only 40.3% in elderly with underlying conditions including heart disease, lung disease, asthma, diabetes, liver disease and smoking [145].

Concerning CKD patients, no RCTs on influenza vaccine efficacy could be found. Efficacy in this group is extrapolated from serology data, for which hemagglutination inhibition (HAI) titres ≥1:40 are assumed to be about 50% protective, and ranges from 36.8% to 94.7% depending on the influenza strain [146]. There is insufficient data to assess efficacy of pneumococcal vaccination in CKD patients. Concerning COPD patients, a RCT reported influenza vaccine efficacy of 76% in preventing acute respiratory infection [147]. A recent Cochrane meta-analysis showed that pneumococcal vaccination (conjugate or polysaccharide vaccine) is effective in reducing CAP among COPD patients, but could not find evidence of reduction in confirmed pneumococcal pneumonia [148]. On the contrary, another review remained inconclusive as the included studies yielded contradictory results [149]. Concerning diabetic patients, a meta-analysis based on observational studies in diabetic patients of working age (18–64 years) found that influenza vaccination prevented all-cause hospitalization with a pooled vaccine efficacy of 58% and hospitalization due to influenza or pneumonia with a vaccine efficacy of 43 %, but no effects on all-cause mortality and influenza-like illness (ILI) were observed [150]. In contrast, five studies included in another recent meta-analysis reported that seasonal influenza vaccination was effective in preventing all-cause mortality, but especially among those aged ≥65 years [127]. No RCT on pneumococcal vaccination in

diabetes patients was found, but a population-based retrospective cohort study found reduced risks of IPD, respiratory failure, and shorter length of hospitalization [151]. No RCT could be found that estimated influenza or pneumococcal vaccine efficacy in patients with cystic fibrosis. Efficacy studies in patients with cardiovascular disease showed an effect of influenza vaccination on cardiovascular mortality, but no effect on laboratory-confirmed influenza. No studies on pneumococcal vaccine efficacy in patient with cardiovascular disease were found. Vaccine efficacy in HIV patients depends on the severity of immunosuppression. For example, a recent systemic review and meta-analysis demonstrated that PCV13 effectively prevents IPD. However, HIV-infected children who are severely immunocompromised are not protected against IPD. Regarding influenza vaccination in HIV patients, a meta-analysis found that influenza vaccines were effective, albeit only moderately. Due to methodological constraints they could, however not assess the influence of ART [144]. Efficacy studies in SOT patients are scarce and have limited patient numbers [152]. Hence, recommendations are mostly based on low sample size studies and data from immunocompetent persons.

3.3.1 Efficacy of HPV vaccination in HIV and transplant patients.

Data on HPV vaccine efficacy in HIV and SOT patients are lacking. Some concerns about the efficacy in HIV patients exist given the potentially higher HPV exposure. A recent double-blind placebo-controlled randomized trial of the qHPV-vaccine in HIV-positive adults (82% MSM) aged over 27 years with high current and prior HPV-infections was stopped early due to futility [153]. They found an efficacy of only 22% against persistent anal infection or anal HPV detection, 0% against anal high-grade squamous intraepithelial lesions on biopsy and 88% against persistent oral infection, which should however be interpreted with caution given the wide 95% confidence interval (2-98%). Based on these results, the authors concluded that that they did not support HPV vaccination for the prevention of anal HPV infection or anal HSIL in this population, but that there might be a role for HPV vaccination in the prevention of oral HPV infection. Since it is known that HPV vaccination does not improve clearance of HPV infection and that prior exposure to HPV decreases vaccine efficacy,[88,154] a possible explanation for this low efficacy could be the baseline seroprevalence, which was as high as 60%, 40%, 50% and 30% for HPV 6,11,16 and 18 respectively. Hence, HPV vaccination might still be beneficial for not yet acquired HPV-types in HIV patients but efficacy studies are needed to prove this. It might as well be that the 9HPV vaccine is more efficacious as it covers more HPV types, but studies have not yet been performed. Nevertheless, preference should still be given to vaccination before sexual onset. For SOT patients, there are no efficacy data available.

3.4. Additional advantages of vaccination in at-risk groups

Although vaccines might not always be fully protective to all at-risk groups, which might either be through primary or secondary vaccine failure, they are beneficial as they mitigate disease severity or avoid deterioration of chronic diseases. For example, a recent study from the United States showed that up to date pertussis vaccination reduces the probability of hospitalization by 66% in 1- to 10 years-olds [155]. Also, the risk of complications caused by diphtheria toxin has shown to be inversely proportional to the number of received diphtheria vaccines [156]. Furthermore, seasonal influenza vaccination has been associated with lower hospitalization rates due to acute myocardial infarction, heart failure, and pneumonia/influenza [127]. Influenza vaccination is also assumed to reduce acute myocardial infection with estimates of the efficacy of ranging from 15% to 45% [157]. A study in patients with a cardiovascular disease showed that influenza vaccination reduced the probability of all-cause and cardiovascular death with 18% [158]. This is comparable to the efficacy of accepted routine coronary prevention actions such as smoking cessation (32-43%), statins (19-30%) and antihypertensive therapy (17-25%). In terms of diabetic patients, multiple logistic regression analysis in a case-control study estimated that influenza vaccination reduced hospital admissions by 79% during two epidemics [159]. In diabetic patients aged over 65 years, influenza vaccination has shown to prevent all-cause mortality with a vaccine efficacy of 38% and all-cause hospitalization with a vaccine efficacy of 23% [150]. Furthermore, influenza vaccination has shown to prevent 59%–78% of asthma attacks leading to emergency visits and/or hospitalizations [160]. A placebo-controlled study also found less COPD exacerbations in influenza vaccinated COPD patients compared to unvaccinated at-risk patients [126]. Influenza vaccination also has a beneficial effect on transplant patients. During an influenza outbreak in a kidney transplant unit, none of the vaccinated patients developed severe disease, whereas several cases of respiratory failure and death occurred among the unvaccinated patients [84].

Better clinical outcomes after pneumococcal vaccination have also been described. For example, PPSV23 vaccination has been associated with faster resolution of symptoms, decreased duration of hospital stay and reduced severity and mortality in patients hospitalized with pneumonia [121]. A recent Cochrane review also showed that pneumococcal vaccination reduced COPD exacerbations [148].

3.5. Safety of vaccines in at-risk groups

Inactivated vaccines are generally considered safe in all at-risk groups. In terms of SOT recipients, there is always the fear of an acute graft rejection due to an inflammatory response, as seen in the case of acute infections. This was suggested by some studies, but disputed by many others [84]. The so far largest pediatric study of this kind assessed transplant outcomes in pediatric renal transplant patients who received an influenza vaccine in the first year after transplantation. They found that vaccinated children had lower risk of mortality and found no differences in graft survival or rejection between vaccinated and unvaccinated children [84]. Furthermore, it could be suggested that immunosuppression reduces local reactions upon vaccination. Clinical trials on the influenza vaccine and PCV7 vaccine have reported less AEs to vaccination in SOT patients compared to healthy controls [161,162].Similarly, the occurrence of reactions following vaccination decreases with age, as shown for the inactivated influenza vaccine [163,164].

Live vaccines are generally not given to severely immunocompromised patients due to the risk of inducing disease by vaccination. For this reason, safety data on the use of live-vaccines are sparse in these patient groups. However, it is suggested that MMR and varicella vaccination might be safe in certain moderately immunocompromised patients, such as transplant recipients in case of limited immunosuppression, stable disease and no history of graft rejection [165]. In stable pediatric HIV patients in whom the immune functioning is reconstituted with ART use, live vaccines can as well be administered [130]. Safety concerns might also depend on the administration mode, dose and duration of immunosuppressive treatment. For example, when prednisone (corticosteroids) is administered systemically at more than 2 mg/kg per day or at a total daily dose greater than 20 mg per day for more than 14 consecutive days, more concerns raise compared to topical application corticosteroids at a lower dose [165].

3.5.1. Safety of HPV vaccination in HIV and solid organ transplant patients

Regarding HIV patients, some questions have been raised about the influence of vaccines on the HIV viral load and CD4-level. However, multiple studies have shown that the bivalent and qHPV vaccines are safe and well tolerated in HIV-infected people, and could not find any effect on the CD4-level or HIV viral load [166,167]. Studies with the nine-valent vaccine have not yet been performed.

With regards to the SOT population, only four studies have reported safety data for the qHPV vaccine [139–142]. No studies were done with the bivalent or nine-valent vaccines. Three of those studies reported no safety concerns and one was stopped prematurely due to acute kidney rejection in 6 out of 14 participants, but a relationship with the vaccination could not be determined. All these studies had small sample sizes and remained inconclusive about safety. Since HPV vaccines are inactivated vaccines, they are considered safe.

4. Vaccination as an indirect tool to prevent infectious diseases

In addition to providing direct protection to the vaccinated person, vaccination can also indirectly protect non-immune or non-vaccinated persons, when a large proportion of the population is vaccinated. Herd immunity or community immunity refers to the fact that high vaccination uptake in the population reduces circulation and transmission of the disease and as such protects our most vulnerable individuals, those who can't be vaccinated and those who do not respond to vaccination due to underlying disease or treatment. It is estimated that 92-96% of the population must be vaccinated to eliminate measles and pertussis, 84-88% to eliminate rubella and 88-92% to eliminate mumps in Western Europe [168]. For tetanus, no herd immunity can be reached as there is no human to human transmission. As an example of herd immunity, a recent meta-analysis showed that in countries with girls-only HPV vaccination programs, anogenital warts diagnosis also decreased significantly among boys and men [52].

Another strategy that is used to indirectly protect vulnerable individuals is the cocoon strategy. This implies that all close contacts of a vulnerable individual are vaccinated in order to avoid them from being a source of transmission. The cocoon strategy is for example applied for influenza vaccination. It is recommended that all people that live with a person aged ≥65 years or an individual with a chronic condition get vaccinated. This also applies to HCWs who care for vulnerable patients. Indirect protection might especially be beneficial for at-risk patients who might not be immune or who cannot be vaccinated.

4.1. Seroprevalence

Seroprevalence studies assess the proportion of the population that has antibodies against a certain disease. They are essential to assess success of vaccination programs and susceptibility of the population to vaccine-preventable diseases. For vaccine-preventable diseases for which a correlate of protection has been defined, one can also assess the level of protetion in the population. Seroprevalence studies are sparse in Belgium. In 2002, a serosurvey on diphtheria, tetanus, measles, mumps and rubella was done in the context of European seroepidemiology network 2 (ESEN2) [169]. Tetanus titers were only determined in persons aged over 40 as these people were less likely to have been reached by universal vaccination programs. Titers for the other vaccine-preventable diseases were assessed in individuals between the ages of 1 and 65 years. Seroprotective levels were reached in 90.7% for tetanus, in 55% for diphtheria and in 87.6% for rubella. For measles and mumps, seroprevalence of antibodies does not correspond with protection as no correlate of protection is defined, but 96.1% and 89.6% were not seronegative (seropositive or equivocal) for measles and mumps respectively [169]. Van der Wielen et al. assessed seroprevalence of pertussis antibodies in 1993-1994, which was before the introduction of the adolescent pertussis booster dose. Antibodies against PT, PRN and FHA were found in about 70%, 40% and 99% of people between 1 and 65 years of age, respectively [170]. Seroprevalence studies to assess susceptibility in patient populations are sparse. However, Heijstek et al. found lower antibody titers for mumps, rubella, diphtheria and tetanus in children with rheumatoid arthritis compared to healthy age-matched controls [171]. A study among HIV patients, of which less than 5% had a CD4+ count of <200cells/µl found seropositivity of 84% for diphtheria, 51% for tetanus and 1% for pertussis [172]. Seroprevalence data of community at-risk patients have not been performed in Belgium.

4.2. Cocoon strategy: vaccination in healthcare workers

Influenza vaccination has shown to reduce cases of influenza in HCWs, days of influenza-like illness (ILI) and absenteeism from work [103]. In this context, a recent study reported a reduction of 12.9% in working days lost upon a vaccination uptake increase from 5% to 37% [173]. HCW vaccination can also serve as a patient safety measure to reduce the carriage and transmission of influenza. This is valuable as nosocomial infections pose a substantial threat to fragile hospitalized patients and residents of LTCFs. Four clustered randomized controlled trials showed that HCW vaccination reduces all-cause mortality and ILI in patients, even at a suboptimal vaccination coverage [174–177]. These RCTs have been criticized by Cochrane reviews, as being biased. The 2010 Cochrane review stressed the fact that the outcomes of the RCTs concerned all-cause mortality and ILI and not influenza-specific outcomes such as laboratory-confirmed influenza and deaths due to pneumonia. Interestingly, a study found that influenza vaccination does reduce laboratory-confirmed influenza but not the incidence of ILI because the virus is replaced by other respiratory pathogens. This indicates that a reduction of ILI might indeed not be the best means of assessing the effect of influenza vaccination, but that influenza vaccination is effective [178]. Moreover, influenza might as well lead to secondary deaths due to strokes and myocardial infarction, which suggests that all-cause mortality might after all not be such a bad measure to assess vaccine efficacy. Later versions of the Cochrane analysis were also controversial as they excluded one RCT or did not perform a pooled analysis. Given that there is no formal agreement about the efficacy of HCW vaccination, randomized controlled trials that include healthcare institutions with a high influenza vaccination coverage (>80%) on laboratory confirmed influenza would be useful to create unequivocally conclusive evidence.

Other vaccines have also been recommended to HCWs in order to prevent disease in risk patients. In the United Kingdom they have been vaccinating HCWs against pertussis since July 2019 in order to prevent them from being a vector of transmission towards young infants. HCWs who were not vaccinated in the last 5 years and who are in contact with young infants and their mothers were prioritized. With the increase in number of measles cases and measles being one of the most infectious pathogens, a recommendation to vaccinate HCW against measles could also be considered.

5. Vaccine recommendations and programs schedule

Recommendations on the vaccination schedule are made by advisory bodies of public health authorities. The WHO's Strategic Advisory Group of Experts (SAGE) offers immunization advices on global policy and strategies, including vaccination recommendations which are reported in WHO position papers. Furthermore, most industrialized countries have national advisory bodies or National Immunization Technical Advisory Groups (NITAG). Based on the local epidemiolocal situation and scientific evidence about immunogenicity, safety and efficacy of a particular vaccine, NITAGs give advice to national policy makers to enable them to make evidence-based decisions about vaccination programs. Since many countries have their own NITAG, vaccination programs vary greatly among countries. Especially in Europe it has been a matter of debate whether all European countries should have the same European vaccination schedule. In Belgium, the NITAG is a part of the Superior Health Council (SHC), which is the advisory body of the Federal Public Health Service Health, Food Chain Safety and Environment that advises the federal minister of public health. Whereas in most countries, decisions about the implementation of vaccines in the vaccination program are made at the national level, in Belgium this is the responsibility of the communities (i.e. Flemish (North), French (South) and German-speaking (East) Community). In Flanders, the "Flemish vaccination council" (Vlaamse vaccinatiekoepel) advises the regional Flemish minister of health about the recommendations of the SHC, who takes the final decision on implementation of vaccines. The Flemish agency for Health and Care (Zorg en Gezondheid) is in charge of the management of the vaccination program, follow-up of vaccination uptake and promotion of vaccines. Table 2 shows the childhood vaccination schedule as currently recommended in Flanders. All these vaccines, except for the vaccine against the rotavirus, are provided free-of-charge. In practice, vaccines for neonates and infants are offered at well-baby clinics organized by Child & Family (Kind&Gezin) which are also in charge of developmental and parenting follow-up of children up to 2.5 to 3 years of age. At school-age, vaccination is organized by the school health service (Centrum Leerlingenbegeleiding (CLB)), which provides preventive care consultation [179]. In children and adolescents, the general physicians and pediatricians only play a complementary role in the follow-up of vaccination status. For the general population of healthy adults, only 10 yearly diphtheria-tetanus-pertussis vaccination is recommended. For adults, there is no public health system in charge of the follow-up of vaccination, and the vaccination status is followed by general practitioners or occupational physicians. Only the tetanus-diphtheria-acellular pertussis (Tdap) vaccine is provided free of charge to all. Influenza vaccination is provided free-of-charge to all institutionalized elderly. There is a central vaccine register (Vaccinnet) in Flanders (Belgium), in which all vaccines should be registered. However, for vaccines that are not provided free-of charge, this is not yet systemically done.

Table 2: Childhood vaccination schedule in Flanders

Vaccine	8 w	12 w	16 w	12 m	15 m	7 y	10 y	12 y	14 y
Diphtheria									
Tetanus									
Pertussis									
Polio									
Haemophilus influenzae B									
Hepatitis B									
13-valent conjugate pneumococcal vaccine									
Rota			( )*						
Measles/mumps/rubella									
Meningococcus type C									
Human Papilloma virus									

* Three doses are recommended if the pentavalent rotavirus vaccine is used (RotaTeq). If the monovalent vaccine (Rotarix) is used, 2 doses are sufficient. All three doses of the pentavalent vaccine need to be given before the age of 32 weeks and the doses of the monovalent vaccine before 24 weeks of age [180].

6. Special recommendations for at-risk groups

Given the increased risk of developing a severe disease, it is highly recommended that at-risk patients follow the standard vaccination schedule, with some additions or minor adaptations specific to their condition. Firstly, the Belgian NITAG recommends annual influenza vaccination for pregnant women, all persons as of the age of 6 months with chronic diseases, all persons aged ≥ 65 years, all institutionalized people and all children between 6 months and 18 years on long-term aspirin treatment. All children below the age of 9 who receive the vaccine for the first time should receive a second dose with an interval of at least 4 weeks. In addition, to improve indirect protection, influenza vaccination is also recommended for healthcare workers, people living with one of the above-mentioned at-risk groups or with a baby below the age of 6 months.

Secondly, since 2019 the Belgian NITAG recommends a dose of the 23-valent polysaccharide pneumococcal vaccine (PPSV23) to immunocompromised children and children with chronic diseases (diabetes, chronic renal disease, chronic liver disease, chronic cardiopulmonary conditions, cystic fibrosis, chronic neurological disease, anatomical or functional asplenia/hyposplenia, cerebrospinal fluid leaks and thymus disfunction), next to the standardly recommended PCV doses. In terms of adult vaccination, pneumococcal vaccination is recommended for immunocompromised persons, persons with an anatomic of functional asplenia, sickle-cell disease or hemoglobinopathy, patients with a cerebrospinal fluid leakage or cochlear implant and persons with chronic disease (chronic renal disease, chronic liver disease (including alcohol abuse), chronic cardiopulmonary conditions (including smokers),

patients with neurological or neuromuscular conditions and diabetes mellitus) and persons between 65 and 85 years of age. The Belgian NITAG recommends using the 13-valent conjugate pneumococcal vaccine (PCV13), followed by a dose of PPSV23 with an interval of at least 8 weeks, and subsequently a PPSV23 booster every five years in patients with an increased risk of pneumococcal infection [181]. If the PPSV23 vaccine is given first, one should wait one year before PCV13 administration.

Thirdly, the recommendations also include vaccination against hepatitis B for individuals who have not been targeted by universal vaccination or who are not yet immune and have an increased risk of exposure to infected blood, such as healthcare workers, patients with HIV, DM, CKD or SOT.

Fourthly, hepatitis A vaccination is recommended to pediatric and adult patients with HIV, chronic liver disease and cystic fibrosis. Furthermore, the Belgian NITAG recommends vaccination with live attenuated vaccines against measles, and mumps, rubella and varicella, but only for non-immune HIV patients with a CD4-count of at least 200 cells/µl, solid organ transplant candidates, and hematopoietic stem cell transplant patient who are not anymore on immunosuppressive treatment, have no graft versus host disease and are at least two years transplanted. Finally, meningococcal vaccination is recommended for immunocompromised patients with an increased personal or epidemiological risk, and HPV vaccination has been recommended for adult immunocompromised patients, including transplant and HIV patients since 2017.

7. Vaccination goals for at-risk patients set by public health authorities.

Vaccination goals set for at-risk patients especially concern seasonal influenza vaccination. In 2003, The World Health Assembly, targeted an influenza vaccination uptake in persons aged ≥ 65 years of 50% by 2006 and 75% by 2010. Moreover, an EU target of 75% vaccination coverage by 2014/15 in the older age groups, and other at-risk groups was set by the Health Council of European union. They also recommended to improve vaccination coverage in HCWs by 2014/15. In 2012, a Flemish action plan was published with the aim of improving the burden of vaccine-preventable disease by 2020. In terms of at-risk patients, it was recommended that at least 75% of persons ≥65 years and 50% of at-risk groups for influenza complications < 65 years would be vaccinated against seasonal influenza annually. Another target was that 80% of HCWs would be vaccinated against seasonal influenza.

For other vaccines, no target has been specifically set for at-risk groups or the targets for the general population apply. For example, the global vaccine action plan, which was approved by the World Health Assembly in 2012, targets to equally widen the benefits of vaccination to all people, including clinical at-risk groups for infectious diseases. In this action plan it was recommended that coverage of target populations should reach at least 90% at the national level for all vaccines in national immunization programs by 2020 [182]. The European vaccine action plan is a regional interpretation of the global vaccine action plan. It targeted that 48 out of 53 countries (90%) had to achieve ≥ 95% coverage with three doses of DTP-containing vaccine at the national level [183]. The WHO developed a global measles and rubella strategic plan, in which they targeted to achieve 95% vaccination coverage for both MMR doses by 2020 [184]. Moreover, WHO European region's targets for measles elimination state that seronegativity should not occur in more than 15% of the children between 2 and 4 years, 10% of children between 5 and 9 and 5% of older children and adults [185]. In accordance with international guidelines,

Belgium has a national action plan since 2003 to eliminate measles and rubella. This plan implies to reach an MMR vaccination coverage of at least 95% for both doses in the general population [186]. Concerning HPV vaccination, in 2018, the director-general of the WHO called for elimination of cervical cancer and for action to end the suffering caused by cervical cancer [21]. It is aimed to reduce cervical cancer incidence to less than 4 in 100 000 by reaching an HPV vaccination coverage of 90% in girls by the age of 15, by screening 70% of women twice during their life and by treating 90% of the precancerous lesions.

8. Vaccination coverage in at-risk groups

8.1 Overview of vaccination coverage in at-risk groups

Vaccination status in at-risk groups is often not monitored. Available studies are mostly limited to a particular vaccine in a certain at-risk group. Unfortunately, these studies often show a vaccination uptake in clinical at-risk groups below the desired level. Studies on the documented uptake of recommended vaccines in pediatric or adult at-risk groups have not yet been performed in Belgium.

Tdap vaccination recommendations are the same as for the general population. A self-reported vaccination coverage of 62% was observed in the general Belgian population in 2008 [187]. Other European countries reported 61-74% [188].

The WHO European region reported influenza vaccination coverage rates of mostly below 40% for persons with chronic illnesses in 14 countries that were able to report vaccination coverage [189]. In 2014/2015, only Scotland (United Kingdom) reached the WHO and European Council's goal to achieve 75% coverage in elderly [189]. Data from the Belgian national health survey in 2013 showed a self-reported coverage of 50% in at-risk patients. A study in the United States reported an influenza vaccination coverage of 41% in diabetic patients and another American study reported a vaccination coverage of 67% in people aged ≥65 years [72]. Similarly, a Spanish study reported a self-reported vaccination uptake of 40% in high-risk patients (diabetes; cancer; chronic respiratory disease; chronic heart disease and cerebrovascular disease) [190].

Self-reported pneumococcal vaccination coverage was 8% in at-risk groups in the Belgian national health survey of 2013 [191]. Other studies report coverages of mostly below 50% that range from 7% in Italy to 50% in the immunocompromised in the United states and up to 60% in high-risk groups in Catalonia (Spain) [192–195]. A recent study based on databases of 2000 French general practitioners, found a pneumococcal vaccination coverage of 8.2% in COPD patients, 2.4% in diabetic patients, 9.1% in congestive heart failure patients and 13.5% in HIV-infected patients [196].

A study in the United States observed an HBV vaccination coverage of 42% in high-risk patients between the age of 18 and 45 years [197]. Another study in the US observed an uptake rate of 27% and a study in Spain an uptake rate of 73% for patients with renal disease [195,198]. For HIV patients, a French study found a vaccination uptake of 62% (at least one dose) [199]. In terms of diabetic patients, an American study found that no patients were vaccinated against HBV [72].

Regarding HPV vaccination, a study in adolescent cancer survivors showed that only about 20% received one dose of an HPV vaccine [200]. A French study based on vaccination data in vaccination booklets found a vaccination coverage of 29% in adolescents with chronical conditions [190].

Healthcare workers

Despite the recommendations and the known advantages of immunizing HCWs, coverage rates remain generally low across Europe, and range from 14% in Poland to 45.6% in England according to a recent review [201]. As part of the underlying idea of HCW vaccination is patient protection, some employers in the United States decided to make it a mandatory condition for employment. This led in most cases to a vaccination uptake of more than 90% [202]. However, due to ethical concerns, mandatory vaccination is not generally accepted.

8.2. Reasons for low vaccination uptake in at-risk groups

In general, the care of at-risk patients is often demanding and complicated resulting in preventive vaccination being overlooked during follow-up of care consultations or postponed. Reasons for non-vaccination in patients concern misconceptions, a lack of awareness of the importance of vaccination, fear of adverse outcomes or illness after vaccination and practical reasons (inconvenience, price) [72]. A current issue might be that risk-patients are closely followed by a specialist and therefore consult their general practitioner in a lesser degree, whereas the specialist does not approach the patient about vaccination, as he/she considers vaccination to be the task of the general practitioner [72]

In the adult population, an important determinant of vaccination against influenza and pneumococcal disease is *age*. Multiple studies on vaccination coverage of influenza and pneumococcus describe higher coverages in elderly than in younger people, for whom the vaccines are recommended as well [193,194,203,204]. For example, an Irish study found a vaccination coverage of 28% against influenza and 16% against pneumococcus in the younger at-risk population (18-65 years) and 60% against influenza and 36% against pneumococcal disease in persons aged over 65 years [193]. A similar difference was observed in a study in which they assessed the influenza vaccination coverage in 11 European countries [205]. This study suggested that most countries are more devoted to elderly vaccination compared to vaccination of younger at-risk groups. Another important factor that has been widely associated with vaccination uptake is a *recommendation by a physician* [193,203]. Giese *et al*. reported not considering vaccination to be necessary as the main reason for non-vaccination against influenza in persons 18 to 65 years of age for whom influenza vaccination was recommended [193]. Furthermore, a Spanish study linked *unhealthy lifestyle* to under-vaccination in COPD patients [206]. Other studies also report higher coverages among patients with lung diseases [193,194]. As influenza and pneumococcal disease might lead to disease exacerbation of COPD, it might be that more attention is paid to their vaccination status. Likewise, higher vaccination coverage against influenza in patients with cardiovascular disease is seen, likely since influenza has been associated with the worsening of pre-existing heart disease [192–194].

Factors that have been associated with HBV vaccination uptake are a high income, younger age, higher education, follow-up with an experienced physician and higher CD4 count in HIV patients [197,199].

Predictors of non-vaccination against HPV in a study in adolescent cancer survivors included a lack of recommendation by physician, perceived lack of insurance coverage for HPV vaccine, male sex, concerns about cost, logistics, safety, or vaccines in general, and younger age [200]. A French study in adolescents with chronic conditions found associations between vaccination and age and low education of the father [190].

Healthcare workers: influenza vaccination

Influenza vaccination uptake has been associated with male gender, age (≥40 years), higher education, having chronic illness, time working in healthcare sector, day shifts, being a nurse versus a nursing assistant, and being a physician versus nursing assistant [207,208]. Furthermore, it has been associated with behavioral determinants such as perceived susceptibility, perceived severity of disease, trust in the effectiveness of the vaccine, disagreement with myths, knowledge about the recommendations and guidelines and social influence. Lastly, organizational determinants such as a convenient timing of vaccination play a role [207,208]. The major reason for vaccination, on the other hand, is most frequently self-protection, rather than protection of patients.

8.3. Interventions to improve vaccination coverage

Vaccination coverage can be improved by vaccination campaigns in which the determinants of low vaccination coverage and barriers towards vaccination are addressed. The guide to Tailoring Immunization Programs (TIP) from WHO is a useful tool to assess these determinants and barriers and implement evidence-based interventions to counter them. Studies on the introduction of well-organized multi-intervention vaccination campaigns showed a significant increase in vaccination uptake in at-risk patients [209,210]. Recently, studies have shown that reminder applications and pharmacy-based immunization services are useful tools to increase vaccination coverage in at-risk groups [211].

Healthcare workers

Different strategies to improve the vaccination coverage among healthcare workers have been explored, including extensive communication, education, free-of-charge vaccination, and easy access to the vaccine. [212,213]. Furthermore, some healthcare institutions have introduced a vaccinate-or-mask policy, in which non-vaccinated HCWs are required to wear a mask during the influenza season. This has shown to be a mind-changing factor for some HCWs, but offered an alternative for vaccination in other HCWs [214]. Mandatory vaccination is not generally accepted in Europe since many ethical concerns are raised. The duty not to harm or infect patients conflicts with the freedom to decide whether or not to have the vaccine. As long as this debate remains ongoing, well-prepared campaigns that include multiple interventions are the best available means to increase vaccination coverage. It has been demonstrated that multi-intervention strategies are the most effective in increasing vaccination coverage [212]. Reviews on intervention programs showed that a combination of education, promotion and improved access is more effective than a single intervention programs [202,212,213]. A study found that vaccination coverage was at least twice as high among HCWs who reported at least two interventions at their workplace, compared to no intervention [215]. A recent intervention study in Italy found an increase in HCW influenza vaccination coverage from 5.2% (2014/2015 season) to 37.2% (2018/2019 season) after introduction of vaccine administration within hospital units, dedicated web

pages on social media and web site, and mandatory compilation of a dissent form for those HCWs who refused vaccination [173]. The World Health Organization (WHO) Regional office for Europe recommends tailoring such seasonal influenza immunization programs in an evidenced-based way to a specific setting [216]. In this context, HPRO immune, a project funded by the European Commission's Directorate General for Health and Consumer Affairs Public Health Program, first assessed barriers to vaccination and subsequently developed a toolkit for the organization of vaccination campaigns [217].

9. References

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CHAPTER 2: Objectives

With this PhD project, we aim to increase the understanding of direct and indirect protection induced by vaccination in at-risk patients. Knowledge about epidemiology, immunology, and concerns about vaccines and vaccination in at-risk groups, will give insight in possible issues in the current vaccination policy with regards to at-risk patients. It will offer important information to enable tailoring vaccination programs and vaccines to the requirements of at-risk patients who have the greatest need of protection against vaccine-preventable infectious diseases.

PART I: Direct protection

1) Determination of the immunogenicity and safety of a 9-valent HPV-vaccine in HIV-infected individuals and solid organ transplant recipients
2) Determination of the vaccination coverage of recommended vaccines in adult patients with chronic diseases and exploration of determinants of incomplete vaccination.
3) Determination of seroprevalence of antibodies against diphtheria, tetanus and pertussis in adult patients with chronic diseases.
4) Determination of seroprevalence of antibodies against measles, mumps, rubella, diphtheria, tetanus and pertussis in children with chronic diseases.

PART II: Indirect protection

5) Determination of the attitude of healthcare workers towards seasonal influenza vaccination, self-reported vaccination coverage in health care institutions and to explore determinants and reasons for non-vaccination
6) Evaluation of the usefulness of an instruction manual for the organization of influenza vaccination campaigns in long-term care facilities and its impact on vaccination uptake, attitudes of health care workers towards influenza vaccination and reasons for vaccine acceptance.

CHAPTER 3: Immunogenicity and safety of the nine-valent HPV vaccine in solid organ transplant and HIV patients

Manuscript in preparation

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Abstract

Background

The burden of human papilloma virus (HPV) disease in HIV-infected persons and solid organ transplants (SOT) recipients is high. Studies with a quadrivalent HPV vaccine (HPV types 6/11/18/16) show that the immunogenicity is good in HIV patients but suboptimal in SOT patients. In the current study we investigated the immunogenicity and safety of a nine-valent HPV (9vHPV) vaccine (HPV types 6/11/16/18/31/33/45/52/58) in patients with HIV and recipients of a kidney, lung or heart transplant.

Methods

This is a phase III investigator-initiated study in 100 HIV patients (age: 18-45 years) and 171 SOT patients (age: 18-55 years). Three doses of 9vHPV vaccine were scheduled at day 1, month 2 and month 6. Seroconversion rates to all 9vHPV types and geometric mean titers (GMTs) were assessed at month 7. Safety of the vaccine was followed up to Month 7.

Results

All HIV patients seroconverted for all HPV types, but seroconversion ranged from 46% for HPV45 to 72% for HPV58 in SOT patients. Seroconversion rates were particularly low in lung transplant for HPV18 (38%), HPV31 (43%) and HPV45 (32%). GMTs ranged from 180 to 2985 mMU/ml in HIV patients and from 17 to 170 mMU/ml in SOT patients, depending on the HPV type. The 3-dose regimen with 9vHPV vaccine was well tolerated in both patient groups. Most reported adverse events (AEs) occurred at the injection site and included pain, swelling and erythema in 67.7%, 7.1% and 10.1% of HIV patients and 54.7, 8.2% and 5.9% of SOT patients, respectively. Injection site AEs were mostly mild or moderate in intensity. None of the reported serious adverse events were deemed vaccine-related. No patients died during the study.

Conclusion

Immunogenicity of the 9vHPV vaccine is high in HIV patients but suboptimal in SOT patients. The vaccine is safe and well tolerated in both patient groups.

Keywords: Human papilloma virus, nine-valent vaccine, HIV, solid organ transplantation

Introduction

Human papilloma virus (HPV) is the most common sexually transmitted disease and causes about 5% of all cancers worldwide. HPV is a necessary cause of cervical cancer and causes 90% of anal cancers, 60-90% of vaginal cancers, 40-50% of vulvar and penile cancers and lower numbers of oropharyngeal and mouth cancers [1]. Up to 80% of women get infected with HPV during their lifetime, with the highest incidence 3 to 4 years after sexual onset. The risk of infection in men starts shortly after sexual debut and remains high throughout their life. In order to induce malignancies, persistent HPV infection is needed. Fortunately, incidental HPV infections in the general population are usually cleared within 12-24 months [2].

Among HIV-infected persons, HPV tends to persist longer and leads more frequently to genital warts and HPV-related cancers [3,4]. Moreover, such cancers are often more aggressive and more frequently recurring, and genital warts are often refractory to treatment [3,4]. A meta-analysis reported standardized incidence rates as high as 5.8 for cervical cancer, 4.4 for penile cancer, 6.5 for vaginal cancer, 28.8 for anal cancer, 2.3 for oropharyngeal cancer in HIV-positive patients compared to the general population [5]. Likewise for solid organ transplant (SOT) patients, this meta-analysis reported standardized incidence rates as high as 2.1 for cervical cancer, 15.8 for penile cancer, 22.8 for vaginal cancer, 4.8 for anal cancer, 3.2 for oropharyngeal cancer compared to the general population [5]. SOT patients generally take a combination of immunosuppressive drugs to avoid graft rejection: anti-metabolites (mycophenolate mofetil or azathioprine), calcineurin inhibitors (tacrolimus or cyclosporine), and corticosteroids (prednisone) [6]. Immunosuppressive drugs interfere with cellular immunity and counter the clearance of HPV infection, which increases the risk of persistent HPV infection and development of HPV-related disease [7].

So far, three preventive HPV vaccines received marketing authorization: a bivalent vaccine against HPV types 16 and 18, a quadrivalent vaccine (qHPV) against HPV types 6/11/16/18 and a 9vHPV vaccine (9vHPV) against HPV types 6/11/16/18/31/33/45/52/58. Compared to the qHPV vaccine, the 9vHPV vaccine contains five additional Virus-Like-Particles (VLPs) of oncogenic HPV types and has the additional benefit of increasing the coverage of cervical cancers from 70% to 90% [8]. Joura et al. found 96.6% 9vHPV vaccine efficacy against HPV disease caused by HPV31/33/45/52/58 and antibody titers of anti-HPV6/11/16/18 that were non-inferior to those induced by the qHPV vaccine, for which efficacy of >95% was proven [9–11]. Other clinical trials on the 9vHPV vaccine in men (16 to 26 years of age) as well as in girls and boys (9 to 15 years of age) demonstrated antibody titers that were non-inferior to those in women between 16 and 26 years of age, which allows bridging of efficacy data from women to men and boys and girls [11–14].

Many high-income countries recommend HPV vaccination in young girls and some in boys as well. In addition, the Advisory Committee on Immunization Practices (ACIP) of the United States recommends HPV vaccination for immunocompromised individuals (including those with HIV infection) up to the age of 26 years [15]. The Belgian National Immunization Technical Advisory Group (NITAG) recommends vaccination with the 9vHPV vaccine for adult immunocompromised patients, including transplant and HIV patients since 2017, without age specification [16]. Even though HPV vaccination has shown to be

very immunogenic and efficacious in preventing HPV infections in healthy populations, studies in HIV and SOT patients are scarce and none of these have yet evaluated the 9vHPV vaccine. The few published studies on the bivalent or qHPV vaccine showed suboptimal immunogenicity in adult SOT patients but results were better in HIV-infected patients with a reasonable CD4-count (>200 cells/mm²) [17,18]. In the current study, we assessed the immunogenicity and safety of a 9vHPV vaccine in both HIV and SOT patients.

Materials and methods

Study design and population

This is a single center, open-label, investigator-initiated phase III study (protocol V503-044-IC, NCT03525210) in HIV patients and SOT recipients to evaluate the immunogenicity with respect to HPV types 6/11/16/18/31/33/45/52/58 and safety/tolerability of the 9vHPV vaccine (Gardasil®9 (Merck Sharp & Dohme (MSD))). One hundred HIV patients (age: 18-45 years) and 171 SOT (kidney, heart, lung transplant) patients (age: 18-55 years) were enrolled between April 2018 and January 2019 in the outpatient clinic of the University Hospitals Leuven, Belgium (Figure 1). This a tertiary referral hospital in Belgium in which approximately 900 HIV patients and 415 heart, 590 lung and 700 kidney transplant patients were followed at study onset. Of these patients, about 370 HIV, 160 heart transplant, 210 lung transplant and 350 kidney transplant patients were age-eligible. All patients had to be in a stable health condition apart from being infected with HIV or a having a solid organ transplant. Further requirements for inclusion were no history of previous HPV vaccination, positive HPV test, positive Papanicolaou (pap) test or any HPV-related disease. A protocol modification in January 2019 allowed a history of genital warts in HIV patients. In addition, HIV patients were required to have a CD4+ T cell count of at least 200 cells/µl at the latest check-up (< 16 months ago). Organ transplantation had to be performed at least 12 months prior to the first vaccination and SOT patients were also required to have been stable for at least 6 months prior to inclusion (i.e. no acute rejection or other immunological reaction) and could not have a history of genital warts. Patients who received multiple solid organ transplantations were allocated to the group which required the highest dose of immunosuppressive medication (i.e lung>heart>kidney). Signed informed consent was obtained from all participants. The study was approved by the Ethics Committee Research of UZ/KU Leuven, Belgium (S60879).

Vaccine

All patients were scheduled to be vaccinated with three doses of the 9vHPV vaccine which contained 30µg of HPV-6, 40µg of HPV11, 60 µg of HPV16, 40 µg of HPV18, 20 µg of HPV31, 20 µg of HPV33, 20 µg of HPV45, 20 µg of HPV52, 20 µg of HPV58 and 500 µg of amorphous aluminum hydroxyphosphate sulfate adjuvant. The vaccines were administered at day 1, and at 2 and 6 months. Before each vaccination, participants had to be afebrile (oral temperature <37.8°C) for 24 hours. Female participants were instructed to use effective contraception during the course of the study and a pregnancy test (urine test for β-human chorionic gonadotropin) was taken before each vaccination.

Immunogenicity assessment

The primary immunogenicity outcome of this study was seroconversion, which is a change in serostatus of anti-HPV antibodies from seronegative at baseline to seropositive at month 7. The secondary outcome was geometric mean titers (GMTs). Serology testing was performed on serum samples collected at day 1 and month 7 using a competitive Luminex® immunoassay (cLIA), as previously described [19]. Patients were defined as seropositive for anti-HPV 6/11/16/18/31/33/45/52/58 if they had titers above 50/29/41/59/29/22/15/20/15 milli Merck Units (mMU), respectively. Since type-specific antibodies were used to assess antibodies for each HPV-type in the cLIA, titers of HPV-types cannot be compared.

Immunogenicity of the 9vHPV vaccine was assessed using the per protocol immunogenicity (PPI) population. This included all patients who received all three vaccine doses of 9vHPV vaccine within pre-specified acceptable intervals, were seronegative to a particular HPV type at baseline, had serology results based on serum samples collected within pre-specified acceptable day ranges and had no other protocol deviations that could interfere with the subject's immune response to the 9vHPV vaccine as judged by the principal investigator. Data on the all type-specific naïve subjects with serology (ANSS) population are added in supplementary materials. This population included all participants that received all 3 vaccinations with the 9vHPV vaccine, were seronegative to the appropriate HPV type at Day 1, had serology results based on serum samples collected within pre-specified acceptable day ranges for ANSS population (21 to 105 days after dose 3). For the ANSS population, protocol deviations that could interfere with the subject's immune response were not taken into account.

Safety assessment

Patients were observed for 15 minutes after each vaccination for immediate reactions. Solicited systemic and injection site adverse events (AEs) as well as a daily evening temperature were recorded from day 1 until day 5 on diary cards. Other adverse events and serious adverse events (SAEs) were recorded from day 1 until month 7 (one month after the last vaccine). Swelling and erythema were rated based on size as mild (0 to ≤2.5 cm), moderate (>2.5 to ≤5.0 cm) or severe (>5.0 cm). Patients were asked to rate any other AE as mild (noticeable but easily tolerable), moderate (interferes with daily activities) or severe (causes a lot of burden and prevents daily activities). Serious adverse events were defined as any adverse event, regardless of causality with vaccination, that results in death, is life-threatening, results in significant disability, causes hospitalization or prolongs an existing inpatient hospitalization, is cancer, is a congenital birth defect, is associated with an overdose or is any other important medical event. Causality of the AEs and SAEs with respect to the administration of the study vaccine (classified as not related, possibly or probably related) was assigned by the investigator based on timing of the AE and the known safety profile of the 9vHPV vaccine. All patients who received at least one dose of the vaccine and who had safety follow-up data for at least one dose of the vaccine, were included in the safety analysis.

Statistical analysis

A sample size of 100 HIV patients was calculated based on the expectation of having at least 80% seronegative samples for each of the 9 HPV types prior to vaccination. This allowed to estimate an anticipated seroconversion rate of 95-99% for HPV types 6, 11 and 16 with a margin of error of ± 4.8% - 2.2%, and an anticipated seroconversion rate of 90% for HPV type 18 with a margin of error of ±6.6% [20]. Further, a sample size of 170 SOT patients was calculated based on an expected seroconversion rate of 60% and a desired precision of ±7.5% [21].

Seroconversion rates for HPV types 6/11/16/18/31/33/45/52/58 are listed with exact binomial 95% confidence intervals (95%CI). GMTs and 95% confidence intervals (95%CI) were calculated from the logarithm of antibody titers and back-transformed to the measurement scale. Antibody titers below the lower limit of quantification (LLOQ) were replaced by LLOQ divided by two for the calculation of GMTs and associated confidence intervals. The LLOQ was 20/16/20/24/10/8/8/8/8 for HPV6/11/16/18/31/33/45/52/58, respectively. Predictors of seroconversion were assessed with multiple logistic regression in the SOT patients. In the HIV group we analyzed predictors of the log transformed titres with multiple linear regression analysis since all subjects were seropositive after vaccination.

The prevalence of AEs and safety measures is given with an exact Clopper-Pearson 95% CI and compared with historical controls using an exact binomial test for proportions. Since the majority of enrolled patients were male (75%) and the safety profile of the 9vHPV vaccine has been shown to be more dependent on gender than on age [22], historical safety data from males between 16 and 26 were used as comparator [23]. A test probability of 5% was considered statistically significant. All data were analyzed with R version 3.6 (R Foundation for Statistical Computing, Vienna, Austria, 2019).

Results

Patient characteristics

In total 287 patients were screened of whom 271 were enrolled in the study, 100 in the HIV group and 171 in the SOT group (56 with a renal transplant (RTX), 57 with a heart transplant (HTX), and 58 with a lung transplant (LTX)) (figure 1). Table 1 shows the baseline characteristics of the patients in each group. The mean age at the first visit was 38.9 years in the HIV group and 46.7 in the SOT group. In total, 85.0% were male in the HIV group and 69.0% in the SOT group. There were considerably more participants with a non-Caucasian origin (23% African and 9% other) in the HIV group compared to the SOT group (1.2% and 0.6%, respectively).

In the HIV group 8% had history of genital warts. One person from the SOT group had a history of genital warts which was only revealed at visit 2 and was subsequently excluded from the PPI analysis, as this was a violation of the inclusion criteria of the SOT group. Of the HIV patients, 4% had a CD4+ T-cell count of 200-349 cells/µl 16% of 350-500 cells/µl and 80% of >500 cells/µl at time of the first vaccination. Moreover, 99% had plasma RNA levels below the detection limit (<1,6 log copies/ml) and 98% used antiretroviral therapy (ART). In the SOT group, the most frequently used immunosuppressive agents were mycophenolate mofetil (90.1%), tacrolimus (73.1%) and methylprednisolone (48.5%) and most patients used a combination of two or three agents. (table 1).

Overall, 75.0% of HIV patients and 27.5% of SOT patients were seropositive at baseline for at least one vaccine HPV type. In the HIV group, the seropositivity rate for each individual HPV type was more than 15%, except for HPV52 (6%). Seropositivity rates in the HIV group where as high as 34.0% for HPV6, 32% for HPV16 and 28% for HPV18 and 25% for HPV58. In contrast, seropositivity at baseline was below 10% for all HPV types in the SOT group. One HTX patient was seropositive at baseline for all the vaccine HPV-types but had no known history of HPV vaccination.

Table 1: Patient characteristics

	All patients N=271	HIV N=100	Kidney Tx N=56	Heart Tx N=57	Lung TX N=58	All SOT N= 171
Personal data						
Age, median (range)	42 (18-55)	38 (18-45)	47 (22-55)	46 (19-55)	45 (22-55)	46 (19-55)
Male sex, n (%)	203 (74.9)	85 (85.0)	35 (62.5)	46 (80.7)	37 (63.8)	118 (69.0)
Origin, n (%)						
Caucasian	236 (87.1)	68 (68.0)	55 (98.2)	55 (96.5)	58 (100.0)	168 (98.3)
African	25 (9.2)	23 (23.0)	1 (1.8)	1 (1.8)	0 (0.0)	2 (1.2)
Other [a]	10 (3.7)	9 (9.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (0.6)
Women of child-bearing age, n	49	15	15	6	13	34
Hormonal anticonception, n (%) [b]	26 (48.2)	4 (21.6)	8 (45.7)	4 (61.5)	10 (87.0)	22 (62.0)
BMI, kg/m² median (range)	24.4 (15.2-44.9)	24.4 (15.2-42.2)	25.5 (17.0-44.9)	25.2 (16.0-29.1)	22.6 (17.2-33.6)	24.4 (16.0-44.9)
Disease characteristics						
Number of active comorbid diseases, median (range)	3 (0-22)	2 (0-6)	4 (0-8)	2 (0-7)	4 (1-22)	4 (0-22)
Time since HIV diagnosis or transplantation, years (median (range))	7 (1-31)	8 (1-31)	7 (1-30)	8 (1-27)	4 (1-17)	6 (1-30)
HIV						
CD4+ T-cell count, cells/µl (median(range))		737 (208-1419)				
Nadir CD4, cells/µl (median, range)		274 (0-896)				
SOT						
Immunosuppression at baseline, n (%)						
Type						
methylprednisolone			24 (42.9)	3 (5.3)	56 (96.6)	83 (48.5)
Azathioprine			6 (10.7)	3 (5.3)	17 (29.3)	26 (15.2)
Cyclosporine			4 (7.1)	5 (8.8)	4 (6.9)	13 (7.6)
Tacrolimus			44 (78.6)	45 (79.0)	36 (62.1)	125 (73.1)
Mycophenolate mofetil			51 (91.1)	50 (87.7)	53 (91.4)	154 (90.1)
Sirolimus or everolimus			0 (0.0)	6 (10.5)	1 (1.7)	7 (4.1)
number						
One immunosuppressive agent			0 (0.0)	2 (3.5)	0 (0.0)	2 (1.2)
Two immunosuppressive agents			38 (67.9)	55 (96.5)	7 (12.1)	100 (58.5)
Three immunosuppressive agents			18 (32.1)	0 (0.0)	51 (87.9)	69 (40.4)
HPV-related characteristics						
History of genital warts, n (%)	9 (3.3)	8 (8.0)	0 (0.0)	0 (0.0)	1 (1.7)	1 (0.6)
HPV seropositivity at baseline, n (%) [c]						
All 9vHPV types [d]	1 (0.4)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (0.6)
At least one 9vHPV type [d]	122 (45.0)	75 (75.0)	17 (30.4)	15 (26.3)	15 (25.9)	47 (27.5)
HPV 6	51 (18.8)	34 (34.0)	5 (8.9)	9 (15.8)	3 (5.2)	17 (9.9)
HPV 11	24 (8.9)	17 (17.0)	2 (3.6)	4 (7.0)	1 (1.7)	7 (4.1)
HPV 16	42 (15.5)	32 (32.0)	3 (5.4)	3 (5.3)	4 (6.9)	10 (5.8)
HPV 18	42 (15.5)	28 (28.0)	5 (8.9)	5 (8.8)	4 (6.9)	14 (8.2)
HPV 31	26 (9.6)	21 (21.0)	1 (1.8)	4 (7.0)	0 (0.0)	5 (2.9)
HPV 33	27 (10.0)	21 (21.0)	1 (1.8)	3 (5.3)	2 (3.4)	6 (3.5)
HPV 45	22 (8.1)	15 (15.0)	2 (3.6)	4 (7.0)	1 (1.7)	7 (4.1)
HPV 52	16 (5.9)	6 (6.0)	3 (5.4)	4 (7.0)	3 (5.2)	10 (5.8)
HPV 58	32 (11.8)	25 (25.0)	3 (5.4)	2 (3.5)	2 (3.4)	7 (4.1)

N= number of participants in each patient group that received at least one dose of the vaccine
ART = antiretroviral therapy, SOT = solid organ transplantation, Tx= transplantation
[a] Other origin includes people with Asian and Latin-American origin.
[b] Women of childbearing potential who did not use hormonal contraception, used either a barrier method, were not sexually active or patient or patient's partner were sterilized.
[c] Percentage of patients with antibody titers above 50,29,41,59,29,22,15,20 and 15 mili-Merck Units for HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 respectively.
[d] 9vHPV types: HPV type 6,11,16,18,31,33,45,52 and 58

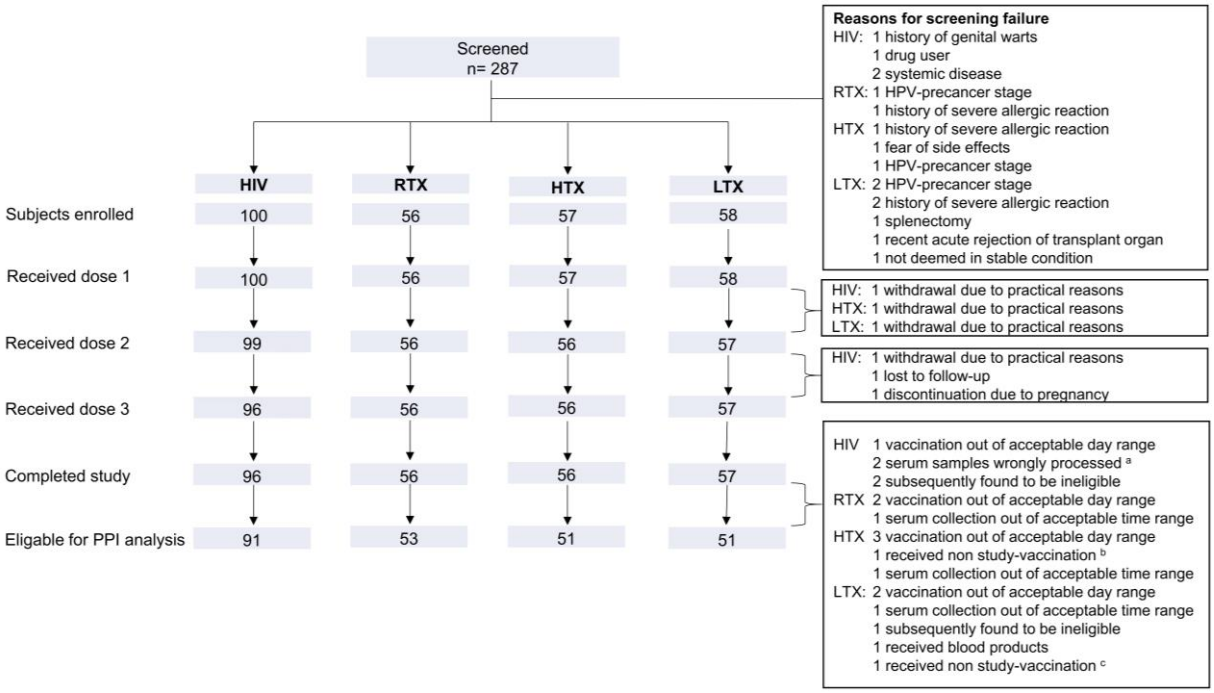

Figure 1: **Study flow for all patients who provided informed consent**

[a] Serum samples were centrifuged at 365 g instead of 1942 g

[b] Received an inactivated vaccine within ±14 days of study vaccination

[c] Received an inactivated influenza vaccine within ±7 days of study vaccination

HIV: human immunodeficiency virus, RTX: renal transplantation, HTX: heart transplantation, LTX: lung transplantation

Immunogenicity

Table 2 shows the GMTs and seroconversion rates of the PPI population. Whereas all HIV patients seroconverted for all HPV types, seroconversion ranged from 46% for HPV45 to 72% for HPV58 in SOT patients. Seroconversion rates were particularly low in lung transplant patients for HPV types 18 (38%), 31 (43%) and 45 (32%). The GMT ranged from 180 to 2985 mMU/ml in HIV patients and from 17 to 170 mMU/ml in SOT patients, depending on the HPV type. GMTs and seroconversion rates of the ANSS population are added in supplementary materials (supplementary table 1)

Table 3 shows the predictors of seroconversion in SOT patients for the PPI population. In HIV patients, seroconversion rates could not be further investigated because they all seroconverted, but significant higher titers were reached in patients with an African origin compared to Caucasians for all HPV type except 6 and 11. There was no clear effect of the CD4 count, except for lower titers with increased CD4-count for HPV45.

In SOT patients, seroconversion rate was significantly higher in women for HPV31 and decreased significantly with higher BMI for HPV6. Moreover, seroconversion was lower for all studied HPV types when the patient received mycophenolate mofetil or tacrolimus, albeit only significant for mycophenolate mofetil. Multiple linear regression of the log transformed titres on month 7 showed similar effects, with some minor differences in significance levels for particular HPV types (data not shown).

Inclusion of data from patients who were seropositive at baseline in the multiple linear regression models with an additional dichotomous variable for seropositivity at baseline showed significantly higher log titers in HIV and SOT patients who were seropositive at baseline. This was significant for all HPV types, except for HPV52 in the HIV group (p=0.6). Month7 GMTs were also 1.2 to 2.6-fold higher in HIV patients and 3.0 to 12.5-fold higher in SOT patients who were seropositive at baseline. In addition, use of mycofenolate mofetil and tacrolimus was inversely associated with log titers for all HPV types and HPV6/16/18/31/58, respectively. This analysis, and description of GMTs at baseline and month 7 in patients who were seropositive at baseline is provided as supplementary data (supplementary table 2 and 3)

Table 2: Month 7 geometric mean titers and seroconversion in the PPI population

	All patients N=271		HIV N=100		Kidney Tx N=56		Heart Tx N=57		Lung Tx N=58		All transplant N=171	
cLIA assay	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml
Anti-HPV 6	202	181 (146-225)	62	831 (679-1016)	49	91 (62-133)	43	127 (87-185)	48	71 (49-102)	140	92 (74-115)
Anti-HPV 11	226	148 (119-184)	76	693 (566-850)	52	63 (42-95)	48	91 (61-135)	50	54 (37-79)	150	67 (54-85)
Anti-HPV 16	212	382 (287-509)	63	2589 (2096-3197)	51	159 (93-271)	49	334 (193-577)	49	93 (55-158)	149	170 (123-234)
Anti-HPV 18	210	158 (132-189)	67	613 (497-757)	49	79 (60-104)	47	119 (88-161)	47	63 (51-78)	143	84 (72-98)
Anti-HPV 31	222	93 (73-117)	70	441 (349-556)	53	44 (28-69)	48	77 (49-122)	51	28 (20-40)	152	45 (35-58)
Anti-HPV 33	224	88 (73-106)	73	317 (263-382)	53	45 (32-63)	49	65 (44-94)	49	36 (26-51)	151	47 (38-58)
Anti-HPV 45	227	38 (31-47)	77	180 (146-223)	52	15 (11-22)	48	28 (19-40)	50	12 (9-16)	150	17 (14-21)
Anti-HPV 52	235	89 (72-109)	85	326 (261-409)	51	40 (28-56)	49	61 (39-95)	50	32 (23-45)	150	42 (34-53)
Anti-HPV 58	220	78 (63-96)	70	255 (207-314)	51	44 (29-66)	50	69 (43-110)	49	29 (19-46)	150	45 (34-58)
cLIA assay	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion m % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)
Anti-HPV 6	202	75.2 (68.7-81.0)	62	100 (94.2-100)	49	61.2 (46.2-74.8)	43	69.8 (53.9-82.8)	48	62.5 (47.4-76.0)	140	64.3 (55.8-72.2)
Anti-HPV 11	226	80.5 (74.8-85.5)	76	100 (95.3-100)	52	67.3 (52.9-79.7)	48	77.1 (62.7-88.0)	50	68.0 (53.3-80.5)	150	70.7 (62.7-77.8)
Anti-HPV 16	212	78.3 (72.1-83.7)	63	100 (94.3-100)	51	70.6 (56.2-82.5)	49	77.6 (63.4-88.2)	49	59.2 (44.2-73.0)	149	69.1 (61.0-76.4)
Anti-HPV 18	210	67.1 (60.3-73.5)	67	100 (94.6-100)	49	46.9 (32.5-61.7)	47	70.2 (55.1-82.7)	47	38.3 (24.5-53.6)	143	51.7 (43.2-60.2)
Anti-HPV 31	222	69.8 (63.3-75.8)	70	100 (94.9-100)	53	56.6 (42.3-70.2)	48	68.8 (53.7-81.3)	51	43.1 (29.3-57.8)	152	55.9 (47.6-64.0)
Anti-HPV 33	224	77.7 (71.7-83.0)	73	100 (95.1-100)	53	67.9 (53.7-80.1)	49	73.5 (58.9-85.1)	49	59.2 (44.2-73.0)	151	66.9 (58.8-74.3)
Anti-HPV 45	227	64.3 (57.7-70.5)	77	100 (95.3-100)	52	42.3 (28.7-56.8)	48	64.6 (49.5-77.8)	50	32.0 (19.5-46.7)	150	46.0 (37.8-54.3)
Anti-HPV 52	235	77.9 (72.0-83.0)	85	100 (95.8-100)	51	66.7 (52.1-79.2)	49	71.4 (56.7-83.4)	50	58.0 (43.2-71.8)	150	65.3 (57.1-72.9)
Anti-HPV 58	220	80.9 (75.1-85.9)	70	100 (94.9-100)	51	72.5 (58.3-84.1)	50	78.0 (64.0-88.5)	49	65.3 (50.4-78.3)	150	72.0 (64.1-79.0)

The per-protocol immunogenicity population included all participants that received all 3 vaccinations with the 9vHPV vaccine within pre-specified acceptable day ranges, were seronegative to the appropriate HPV type at Day 1, had serology results based on serum samples collected within pre-specified acceptable day ranges and had no protocol deviations that could interfere with the subject's immune response to the 9vHPV vaccine as judged by the principal investigator.
N= number of participants in each patient group that received at least one dose of the vaccine
n= number of patients contributing to the analysis
m = number of patients that seroconverted
9vHPV = nine-valent human papilloma virus, CI = Confidence interval, cLIA = competitive luminex immunoassay, GMT = Geometric mean titer, HPV= Human papilloma virus, mMU = milli-Merck unit, PPI= per protocol immunogencity, Tx = transplant

Table 3: predictors of log-transformed titers in HIV patients and seroconversion in transplant patients: PPI population

HIV group	**HPV6**	**HPV11**	**HPV16**	**HPV18**	**HPV31**	**HPV33**	**HPV45**	**HPV52**	**HPV58**
Titers	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)
Female sex (vs male)	0.4 (-0.2;1.1)	0.6 (-0.1;1.2)°	-0.2 (-0.9;0.4)	0.0 (-0.6;0.6)	-0.3 (-1.1;0.5)	0.2 (-0.3;0.8)	-0.1 (-0.7;0.5)	0.4 (-0.4;1.1)	-0.1 (-0.8;0.5)
Age (years divided by 10)	-0.1 (-0.4;0.2)	-0.1 (-0.4;0.2)	-0.2 (-0.5;0.1)	-0.2 (-0.5;0.1)	0.1 (-0.2;0.4)	0.1 (-0.2;0.4)	-0.2 (-0.5;0.1)	0.1(-0.3;0.4)	-0.2 (-0.6;0.1)
Origin									
Caucasian	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
African	0.5 (-0.1;1.1)	0.5 (-0.1;1.1)°	**1.0 (0.4;1.5)*** **	**0.9 (0.4;1.5)** **	**1.4 (0.8;2.0)*** **	**0.7 (0.2;1.2)** **	**0.9 (0.3;1.5)** **	**1.3 (0.6;1.9)*** **	**0.8 (0.2;1.4)** **
Other [a]	-0.3 (-1.0;0.4)	0.0 (-0.7;0.7)	0.4 (-0.3;1.1)	-0.1 (-0.8;0.6)	0.0 (-0.8;0.7)	0.4 (-0.2;1.0)	0.4 (-0.4;1.1)	0.0 (-0.8;0.8)	0.4 (-0.4;1.2)
BMI	0.0 (-0.1;0.1)	0.0 (-0.1;0.1)	0.0 (0.0;0.1)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	**-0.1 (-0.1;0.0)** **	0.0 (-0.1;0.0)
CD4+ T-cell count divided by 10	0.0 (-0.1;0.1)	-0.1 (-0.1;0.0)	0.0 (-0.1;0.1)	0.0 (-0.1;0.1)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	**-0.1 (-0.2;0.0)***	0.0 (-0.1;0.1)	-0.1 (-0.1;0.0)
SOT patients	**HPV6**	**HPV11**	**HPV16**	**HPV18**	**HPV31**	**HPV33**	**HPV45**	**HPV52**	**HPV58**
Seroconversion	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Female sex (vs male)	1.5 (0.6;3.9)	1.1 (0.5;2.6)	1.9 (0.8;5.0)	1.0 (0.4;2.5)	**2.8 (1.2;7.0)***	1.4 (0.6;3.6)	1.0 (0.4;2.3)	1.9 (0.8;4.7)	2.2 (0.9;6.0)
Age (years divided by 10)	0.8 (0.5;1.2)	0.7 (0.5;1.2)	0.8 (0.5;1.2)	0.7 (0.5;1.1)	0.8 (0.5;1.2)	0.9 (0.6;1.4)	0.9 (0.6;1.3)	0.7 (0.4;1.0)°	0.8 (0.5;1.3)
Transplant group									
Kidney Tx	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Heart TX	1.2 (0.4;3.6)	1.4 (0.5;4.1)	1.1 (0.4;3.4)	2.3 (0.8;6.5)	1.7 (0.6;4.5)	0.8 (0.3;2.4)	1.9 (0.7;4.9)	1.0 (0.4;2.9)	1.1 (0.3;3.2)
Lung TX	0.9 (0.2;3.0)	0.8 (0.2;2.6)	0.6 (0.2;1.8)	0.6 (0.2;2.1)	0.5 (0.1;1.5)	0.7 (0.2;2.3)	0.8 (0.2;2.6)	0.6 (0.2;1.8)	1.0 (0.3;3.3)
BMI	**0.9 (0.8;1.0)***	1.0 (0.9;1.0)	1.0 (0.9;1.0)	1.0 (0.9;1.1)	1.0 (0.9;1.1)	0.9 (0.9;1.0)	0.9 (0.9;1.0)	1.0 (0.9;1.1)	1.0 (0.9;1.1)
Years since transplantation	1.1 (1.0;1.1)	1.0 (1.0;1.1)	1.0 (1.0;1.1)	1.1 (1.0;1.1)°	1.0 (1.0;1.1)	1.0 (0.9;1.1)	1.1 (1.0;1.1)	1.0 (1.0;1.1)	1.0 (1.0;1.1)
Immunosuppression at baseline, n [b]	0.5 (0.1;1.6)	0.8 (0.2;2.4)	0.5 (0.2;1.5)	0.6 (0.2;1.7)	0.6 (0.2;1.8)	0.3 (0.1;1.0)°	0.4 (0.1;1.0)°	0.6 (0.2;1.9)	0.4 (0.1;1.3)
Mycophenolate mofetil	**0.1 (0.0;0.4)** **	**0.2 (0.0;0.5)** **	**0.3 (0.1;0.7)***	**0.1 (0.0;0.4)*** **	**0.2 (0.1;0.5)** **	**0.1 (0.0;0.4)*** **	**0.3 (0.1;0.8)***	**0.3 (0.1;0.7)** **	**0.2 (0.1;0.6)***
Tacrolimus	0.7 (0.2;2.7)	0.6 (0.1;2.6)	0.3 (0.0;1.3)	0.6 (0.1;2.4)	0.3 (0.1;1.1)°	0.6 (0.1;2.3)	0.6 (0.2;2.6)	0.6 (0.1;2.2)	0.8 (0.2;3.3)

The per-protocol immunogenicity (PPI) population included all participants that received all 3 vaccinations with the 9vHPV vaccine within pre-specified acceptable day ranges, were seronegative to the appropriate HPV type at Day 1, had serology results based on serum samples collected within pre-specified acceptable day ranges and had no protocol deviations that could interfere with the subject's immune response to the 9vHPV vaccine as judged by the principal investigator.

[a] Other included Asian and Latin-American origin.

[b] Number of immunosuppressive drugs taken by patient.

*b= regression coefficient, TX = transplant, BMI= Body Mass Index, °p< 0.1, *p<0.05, **p<0.01, ***p<0.001.*

Safety

The 3-dose regimen with Gardasil-9® was generally safe and well tolerated in HIV and SOT-patients. A summary of the AEs that occurred within 15 days after vaccination is given in table 4. Over the course of the study, 80.8% of the HIV patients and 74.7% of SOT patients reported at least one AE within 15 days after vaccination. Vaccine-related AEs within 15 days after any dose were reported by 74.7 % of the HIV patients and 68.8% of SOT patients. AEs at the injection site were more common, and reported by 69.7% of HIV patients and 57.6% of SOT patients. This included pain, swelling and erythema, which occurred in 67.7%, 7.1% and 10.1% of HIV patients and 54.7, 8.2% and 5.9% of SOT patients, respectively. Injection site AEs were mostly mild or moderate in intensity. Vaccine-related systemic AEs were reported by 24.4% of HIV patients and 20.6% of SOT patients. Headache was the most prevalent vaccine-related systemic AE and was reported by 9.1% of HIV patients and 8.2% of SOT patients.

SAEs that occurred within 15 days after administration of any dose of vaccine and over the course of the whole study are listed by patient group in table 5. In total, eight SAEs were reported within 15 days after vaccination, all in the SOT group. Over the course of the study, 58 SAEs were reported, of which 54 occurred in the SOT group. The SAEs involved a total of 3 HIV and 28 SOT patients. Within the SOT group, hospitalization due to infection was the most frequent reported SAE (10.6%). None of the serious adverse events were considered to be vaccine-related, and none of the seven study discontinuations were due to adverse events. No patients died during the study. One patient became pregnant after receiving the second dose of vaccine and was removed from the study by the investigator, but the pregnancy resulted in a live birth with no known congenital abnormality.

The safety profile of Gardasil®9 is generally similar to that of healthy historical controls, but injection site reactions were reported less frequently compared to historical controls (69.7 % in the HIV group, 57.6% SOT group and 79.0 % in the historical controls, $p<0.05$ for HIV and, $p< 0.001$ for SOT) (Table 4).

Table 4: Summery of safety and tolerability of nine-valent human papilloma vaccine in HIV and solid organ transplant patients

	All patients		HIV		Kidney transplant		Heart transplant		Lung transplant		All Transplant	
	%	95% CI	%	95% CI	%	95% CI	%	95%	%	95%CI	%	95%CI
Subjects with follow-up, n	269		99		56		56		58		170	
With ≥1 AE[a]+	**77.0**	**(71.5-81.8)***	80.8	(71.7-88.0)93	73.2	(59.7-84.2)°	**64.3**	**(50.4-76.6)****	86.2	(74.6-93.9)	**74.7**	**(67.5-81.0)***
With vaccine-related[b] AEs[a]+	**71.0**	**(65.2-76.4)*****	74.7	(65.0-82.9)°	**64.3**	**(50.4-76.6)****	**60.7**	**(46.8-73.5)*****	81.0	(68.6-90.1)	**68.8**	**(61.3-75.7)*****
Injection site event[c]+	**62.1**	**(56.0-67.9)*****	**69.7**	**(59.6-78.5)***	**46.4**	**(33-60.0.3)*****	**51.8**	**(38.0-65.3)*****	74.1	(61.0-84.7)	**57.6**	**(49.8-65.2)*****
Pain[d]+	**59.5**	**(53.3-65.4)*****	**67.7**	**(57.5-76.7)***	**42.9**	**(29.7-56.8)*****	**50.0**	**(36.3-63.7)*****	70.7	(57.3-81.9)	**54.7**	**(46.9-62.3)*****
Mild	58.4	(52.2-64.3)	64.6	(54.4-74.0)	42.9	(29.7-56.8)	50.0	(36.3-63.7)	70.7	(57.3-81.9)	54.7	(46.9-62.3)
Moderate	10.4	(7.0-14.7)	13.1	(7.2-21.4)	3.6	(0.4-12.3)	8.9	(3.0-19.6)	13.8	(6.1-25.4)	8.8	(5.0-14.1)
Severe	0.0	(0.0-1.4)	0.0	(0.0-3.7)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	0.0	(0.0-6.2)	0.0	(0.0-2.1)
Swelling+	**7.8**	**(4.9-11.7)****	**7.1**	**(2.9-14.0)***	10.7	(4.0-21.9)	7.1	(2.0-17.3)	6.9	(1.9-16.7)	**8.2**	**(4.6-13.4)***
Mild (0 to ≤2.5 cm)	7.1	(4.3-10.8)	6.1	(2.3-12.7)	8.9	(3-19.6)	7.1	(2.0-17.3)	6.9	(1.9-16.7)	7.6	(4.1-12.7)
Moderate (>2.5 to ≤5.0 cm)	1.1	(0.2-3.2)	1.0	(0.0-5.5)	1.8	(0.0-9.6)	0.0	(0.0-6.4)	1.7	(0.0-9.2)	1.2	(0.1-4.2)
Severe (<5.0 cm)	0.4	(0.0-2.1)	0.0	(0.0-3.7)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	1.7	(0.0-9.2)	0.6	(0.0-3.2)
Erythema+	**7.4**	**(4.6-11.2)*****	10.1	(5.0-17.8)	8.9	(3.0-19.6)	**1.8**	**(0.0-9.6)****	6.9	(1.9-16.7)°	**5.9**	**(2.9-10.6)*****
Mild (0 to ≤2.5 cm)	7.1	(4.3-10.8)	10.1	(5.0-17.8)	7.1	(2.0-17.3)	1.8	(0.0-9.6)	6.9	(1.9-16.7)	5.3	(2.4-9.8)
Moderate (>2.5 to ≤5.0 cm)	0.7	(0.1-2.7)	1.0	(0.0-5.5)	1.8	(0.0-9.6)	0.0	(0.0-6.4)	0.0	(0.0-6.2)	0.6	(0.0-3.2)
Severe (<5.0 cm)	0.0	(0.0-1.4)	0.0	(0.0-3.7)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	0.0	(0.0-6.2)	0.0	(0.0-2.1)
Pruritus+	1.5	(0.4-3.8)	1.0	(0.0-5.5)	1.8	(0.0-9.6)	1.8	(0.0-9.6)	1.7	(0.0-9.2)	1.8	(0.4-5.1)
Ecchymosis	0.0	(0.0-1.4)	0.0	(0.0-3.7)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	0.0	(0.0-6.2)	0.0	(0.0-2.1)
Induration	0.0	(0.0-1.4)	0.0	(0.0-3.7)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	0.0	(0.0-6.2)	0.0	(0.0-2.1)
Other local events	7.4	(4.6-11.2)	6.1	(2.3-12.7)	5.4	(1.1-14.9)	8.9	(3.0-19.6)	10.3	(3.9-21.2)	8.2	(4.6-13.4)
All systemic events[a]	**46.5**	**(40.4-52.6)°**	**51.5**	**(41.3-61.7)***	50.0	(36.3-63.7)	28.6	(17.3-42.2)°	51.7	(38.2-65.0)	43.5	(36.0-51.3)
Vaccine-related[b] systemic event+	21.9	(17.1-27.4)	24.2	(16.2-33.9)	17.9	(8.9-30.4)	**10.7**	**(4.0-21.9)***	32.8	(21.0-46.3)°	20.6	(14.8-27.5)
Headache+	8.6	(5.5-12.6)	9.1	(4.2-16.6)	7.1	(2.0-17.3)	5.4	(1.1-14.9)	12.1	(5.0-23.3)	8.2 14	(4.6-13.4)
Pyrexia (≥ 37.8°C)+	1.9	(0.6-4.3)	3.0	(0.6-8.6)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	3.4	(0.4-11.9)	1.2	(0.1-4.2)
Nausea+	1.9	(0.6-4.3)	1.0	(0.0-5.5)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	**6.9**	**(1.9-16.7)***	2.4	(0.6-5.9)
Dizziness	1.1	(0.2-3.2)	3.0	(0.6-8.6)	0.0	(0.0-6.4)	0.0	(0.0-6.4)	0.0	(0.0-6.2)	0.0	(0.0-2.1)
Fatigue+	3.3	(1.5-6.3)	4.0	(1.1-10.0)	1.8	(0.0-9.6)	0.0	(0.0-6.4)	**6.9**	**(1.9-16.7)***	2.9	(1.0-6.7)
Other vaccine-related systemic events[a]	13.8	(9.9-18.5)	14.1	(8.0-22.6)	14.3	(6.4-26.2)	8.9	(3.0-19.6)	17.2	(8.6-29.4)	13.5	(8.8-19.6)

n, number of subjects as treated who received at least 1 dose of Gardasil®9 and had at least 1 follow-up visit for AEs
[a] Day 1-15 following any vaccination visit
[b] As reported by the investigator
[c] Days 1-5 following any vaccination visit
[d] Intensities of pain are defined as follows: mild is an awareness of sign or symptom that can be easily tolerated; moderate is discomfort that causes interference with usual activity; severe is inability to work or do daily activities.
+ tested against reference data of historical controls [23]
° $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 5: Serious adverse events by system organ classes

	All patients n=269		HIV n=99		Kidney Tx n=56		Heart Tx n=56		Lung Tx n=58		All Tx N=170	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Days 1-15 after any vaccination												
Subjects with ≥ 1 SAE	8	(3.0)	-	-	1	(1.8)	2	(3.6)	5	(8.6)	8	(4.7)
Infections and infestations	3	(1.1)	-	-	1	(1.8)	-	-	2	(3.4)	3	(1.8)
Nervous system disorders	1	(0.4)	-	-	-	-	-	-	1	(1.7)	1	(0.6)
Respiratory, thoracic and mediastinal disorders	2	(0.7)	-	-	-	-	-	-	2	(3.4)	2	(1.2)
Cardiac disorders	1	(0.4)	-	-	-	-	1	(1.8)	-	-	1	(0.6)
Vascular disorders	1	(0.4)	-	-	-	-	1	(1.8)	-	-	1	(0.6)
Any time during the study												
Subjects with ≥ 1 SAE	31	(11.5)	3	(3.0)	11	(19.6)	5	(8.9)	12	(20.7)	28	(16.5)
Blood and lymphatic system disorders	-	-	-	-	1	(1.8)	-	-	-	-	1	(0.6)
Cardiac disorders	2	(0.7)	-	-	-	-	2	(3.6)	-	-	2	(1.2)
Gastrointestinal disorders	4	(1.5)	-	-	-	-	-	-	4	(6.9)	4	(2.4)
General disorders and administration site conditions	1	(0.4)	-	-	-	-	-	-	1	(1.7)	1	(0.6)
Immune system disorders	1	(0.4)	-	-	-	-	-	-	1	(1.7)	1	(0.6)
Infections and infestations	18	(6.7)	-	-	8	(14.2)	2	(3.6)	8	(13.8)	18	(10.6)
Injury, poisoning and procedural complications	2	(0.7)	1	(1.0)	-	-	-	-	1	(1.7)	1	(0.6)
Metabolism and nutrition disorders	2	(0.7)	-	-	-	-	-	-	2	(3.4)	2	(1.2)
Musculoskeletal and connective tissue disorders	1	(0.4)	-	-	-	-	-	-	1	(1.7)	1	(0.6)
Neoplasms benign, malignant and unspecified	3	(1.1)	-	-	2	(3.6)	-	-	1	(1.7)	3	(1.8)
Nervous system disorders	1	(0.4)	-	-	-	-	-	-	1	(1.7)	1	(0.6)
Psychiatric disorders	1	(0.4)	1	(1.0)	-	-	-	-	-	-	-	-
Renal and urinary disorders	2	(0.7)	-	-	2	(3.6)	-	-	-	-	2	(1.2)
Respiratory, thoracic and mediastinal disorders	4	(1.5)	-	-	1	(1.8)	-	-	3	(5.3)	4	(2.4)
Social circumstances	1	(0.4)	1	(1.0)	-	-	-	-	-	-	-	-
Surgical and medical procedures	2	(0.7)	-	-	1	(1.8)	1	(1.8)	-	-	2	(1.2)
Vascular disorders	1	(0.4)	-	-	-	-	1	(1.5)	-	-	1	(0.6)

n: number of subjects who received at least 1 dose of Gardasil®9 and had at least 1 follow-up visit for AEs

A subject is counted once within a category and may be counted in > 1 category

Discussion

This is the first study that reports on the safety and immunogenicity of a 9vHPV vaccine in both HIV and SOT patients. All HIV patients seroconverted after vaccination whereas among SOT patients, seroconversion ranged from about 45% to 70% depending on the HPV type. The 9vHPV vaccine was safe and well-tolerated in both patient groups.

All HIV patients seroconverted after 9vHPV vaccination, as was reported in healthy women and men between the age of 16 and 26 years [11–14]. Nevertheless, all participants in the HIV group had a CD4-count over 200 cells/µl and nearly all were on ART and had viral loads below the detection limit, which are factors known to contribute to better immunogenicity in patients with HIV [24]. Compared to healthy young adults, the GMTs are in the same range for HPV types 6 and 11 but slightly lower for HPV types 16/18/31/33/45/52/58 [11–14]. This could be due to the older age of the patients in our study, but confirmation is pending because data after 9vHPV vaccination in healthy adults above the age of 26 are not yet available. Another possible explanation is the *original antigenic sin hypothesis,* which assumes that previous antigenic encounter, hampers the immune response when exposed a HPV type with a substantial amino acid similarity [13]. Evidence for this hypothesis is derived from animal studies and a study with the 9vHPV vaccine in humans previously vaccinated with qHPV [25,26]. This is a relevant issue since 75% of HIV patients were seropositive for at least one HPV type at baseline.

High seroconversion rates were also found in a study with a qHPV vaccine in HIV-infected women aged 13 to 45 years. They found also found high seroconversion rates of 95% to 100% for HPV6, HPV11 and HPV16 and from 85% to 100% for HPV18 when the CD4+ count was above 200 cells/µl.[20] The GMTs in our study were also slightly higher compared to the GMTs reported in that study (i.e. 170 to 1200 mMU/ml, depending on the HPV type).[20] However, GMTs in our study were lower when compared to the 980 to 5050 mMu/ml (depending on the HPV type) found after qHPV vaccination in younger (16–23 years) HIV-positive women on ART in another study [17].

The GMTs were generally higher in HIV patients of African origin compared to Caucasian participants. This is in agreement with findings from a study with the qHPV vaccine in men between the 16 and 26 years [27]. The CD4-count was only inversely associated with GMT of HPV45. The absence of a clear effect in one direction for all HPV types in our study might be due to the inclusion of patients with CD4-count over 200 cells/µl only. The clinical relevance of a lower GMT is, however, not clear since no correlate of protection has yet been defined.

Depending on the HPV type, between 45% and 70% of seronegative SOT patients seroconverted after vaccination with the 9vHPV vaccine. The observed seroconversion rates and GMTs are significantly lower compared to data from 16 to 26 year old healthy adults [11–14]. So far, only four studies have reported the immunogenicity of HPV vaccines in SOT patients, and all concerned the qHPV vaccine [21,28–30]. These studies included only 17 to 47 patients and results were inconsistent. One study assessed seroconversion in adult SOT patients and found 63%, 68%, 63.2% and 52.6% seroconversion for HPV6/11/16/18, respectively [18]. The seroconversion rates of 64%, 71%, 69% and 52%, respectively found with the 9vHPV vaccine in our study, are very similar and immunogenicity of the 9vHPV vaccine thus remains suboptimal in these patients. The seroconversion rates were particularly

low in lung transplant patients. Unsurprisingly, the use of mycophenolate mofetil deteriorated the seroconversion and log titers and the use of tacrolimus decreased log titers of HPV6/16/18/31/58. Similarly, Kumar et. al found that failure to seroconvert was associated with higher serum levels of tacrolimus.[18] We could not test the effect of use of other immunosuppressive drugs in our statistical models due to the lack of a sufficiently large number of observations. Future research should assess whether a supplemental dose of the 9vHPV vaccine in SOT patients would increase immunogenicity in patients who did not seroconvert. Even though the immunogenicity with the 3-dose regimen is suboptimal, we still believe that vaccination of SOT patients is beneficial given the high burden of HPV disease. Moreover, a low humoral immunity does not necessarily imply the absence of protection since cellular immunity may also play a role [31]. Yet, studies are needed to confirm this and the clinical efficacy of vaccination in SOT patients.

Since no complete protection against HPV disease can be guaranteed after vaccination in SOT patients, we advocate for pre-transplant vaccination and additional screening at regular time intervals. A study with the 9vHPV vaccine in girls and young women showed a robust immune response in patients with chronic kidney disease but a suboptimal response in patients after a kidney transplant, which indicates added value of pretransplant vaccination [29]. However, if vaccination was not possible or done before transplantation, the best way to protect these patients is to combine post transplantation vaccination with frequent regular screening.

Importantly, HPV vaccination has no therapeutic effect on HPV infections at the time of vaccination, but can still prevent infection with other HPV-types. This is valuable as for each individual HPV type, at least 65% and up to 95% of the HIV patients and more than 90% of SOT patients were seronegative. Furthermore, the GMTs in our study were almost 3-fold higher in HIV patients and up to 12-fold higher in SOT patients who were seropositive at baseline, which indicates boosting of pre-existing immunity. This is valuable as evidence is growing that HPV vaccination can also prevent re-infection with previously cleared HPV types. Evidence for this comes from the qHPV vaccine clinical trials, in which the vaccine was 67% efficient against HPV 6/11/16/18-related persistent infection, cervical intraepithelial neoplasia or external genital lesions in women who were seropositive but had no indication of current infection at the time of vaccination (HPV DNA-negative) [32].

The 9vHPV vaccine was well-tolerated in both patient groups. The most commonly reported injection side AEs were pain, swelling and erythema, usually mild or moderate in intensity. This is in accordance with data from 9vHPV vaccination studies in healthy individuals [22]. The frequency of injection site AEs was lower in our study compared to healthy men between the age of 16 and 26 [14]. The most frequently reported systemic adverse events were headache (9.1% in HIV and 8.2% in SOT group) and fatigue (4% in HIV and 2.9 % in SOT group), whereas in studies in healthy subjects it was generally headache (11%) and pyrexia (5%). None of the SAEs were considered vaccine-related. Although there were more SAEs reported by SOT patients (16.5%) than in healthy adults (2.3%), the large majority was due to infection, which is likely related to their immunosuppressed state [23].

Some limitations of our study should be addressed. Firstly, we included no healthy control group and thus compared our data with historical controls with a different profile with respect to age and gender,

both of which might influence immunogenicity and safety. However, the controls from previous clinical trials used a similar protocol and also used the same technology for antibody measurement (i.e. cLIA), which supports a comparison with these groups. Secondly, we only included HIV patients with a CD4 count above 200 cells/µl which hampers extrapolation of results on safety and immunogenicity to HIV patients with lower CD4-counts, detectable viral load or patients not on ART. Thirdly, we did not record whether the participating men were MSM. This information would be interesting as previous studies found lower GMTs in MSM compared to heterosexual men [13]. The reason for this phenomenon is not understood, but one suggests that have higher previous encounter with HPV in MSM, as seen from higher proportion baseline seropositivity, hampers the immune response upon a subsequent encounter. Fourthly, we could not assess the influence of specific immunosuppressive agents due to limitations of the statistical models and limited sample size. Finally, long-term immune responses and vaccine efficacy in both groups remains to be assessed in future studies.

We conclude that the immunogenicity of the 9vHPV vaccine is excellent in HIV patients but suboptimal in SOT patients. The vaccine is safe and well tolerated in both patient groups. Given the high burden of HPV disease in HIV and SOT patients, the 9vHPV vaccine is beneficial because it covers a broad range of HPV types. Vaccination with the 9vHPV vaccine, preferably pretransplant in SOT patients, in combination with regular screening is proposed as a key strategy to reduce the burden of HPV disease in HIV and SOT patients.

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Supplementary material chapter 3

Supplementary table 1: Month 7 geometric mean titers and seroconversion in the ANSS population

	All patients N=271		HIV N=100		Kidney Tx N=57		Heart Tx N=57		Lung Tx N=57		All transplant N=171	
cLIA assay	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml	n	GMT (95% CI), mMU/ml
Anti-HPV 6	215	183 (149 - 226)	64	842 (691 - 1027)	51	97 (67 - 142)	47	143 (99 - 208)	53	66 (47 - 94)	151	96 (77 - 119)
Anti-HPV 11	241	149 (121 - 184)	80	697 (574 - 847)	54	68 (45 - 101)	52	98 (67 - 143)	55	51 (36 - 73)	161	69 (56 - 87)
Anti-HPV 16	224	380 (287 - 503)	65	2570 (2093 - 3157)	53	176 (103 - 299)	53	360 (213 - 610)	53	83 (50 - 138)	159	174 (127 - 238)
Anti-HPV 18	223	157 (133 - 187)	69	602 (490 - 739)	51	83 (63 - 110)	51	125 (93 - 167)	52	62 (51 - 75)	154	86 (74 - 101)
Anti-HPV 31	238	93 (75 - 116)	75	424 (339 - 531)	55	47 (30 - 73)	52	80 (52 - 124)	56	28 (20 - 38)	163	46 (36 - 59)
Anti-HPV 33	238	87 (73 - 104)	76	308 (257 - 369)	55	48 (34 - 66)	53	67 (47 - 96)	54	35 (25 - 48)	162	48 (40 - 59)
Anti-HPV 45	243	38 (31 - 47)	82	180 (147 - 220)	54	16 (11 - 23)	52	30 (21 - 42)	55	11 (8 - 15)	161	17 (14 - 21)
Anti-HPV 52	248	90 (74 - 110)	90	328 (263 - 408)	53	42 (30 - 59)	52	62 (41 - 96)	53	31 (23 - 44)	158	43 (35 - 54)
Anti-HPV 58	235	78 (63 - 96)	74	257 (211 - 313)	53	46 (31 - 68)	54	71 (46 - 110)	54	28 (19 - 42)	161	45 (35 - 58)
cLIA assay	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)	n	Seroconversion % (95%CI)
Anti-HPV 6	215	74.9 (68.5-80.5)	64	100 (94.4-100)	51	62.7 (48.1-75.9)	47	72.3 (57.4-84.4)	53	58.5 (44.1-71.9)	151	64.2 (56.0-71.9)
Anti-HPV 11	241	80.5 (74.9-85.3)	80	100 (95.5-100)	54	68.5 (54.4-80.5)	52	78.8 (65.3-88.9)	55	65.5 (51.4-77.8)	161	70.8 (63.1-77.7)
Anti-HPV 16	224	78.1 (72.1-83.4)	65	100 (94.5-100)	53	71.7 (57.7-83.2)	53	79.2 (65.9-89.2)	53	56.6 (42.3-70.2)	159	69.2 (61.4-76.3)
Anti-HPV 18	223	68.2 (61.6-74.2)	69	100 (94.8-100)	51	49.0 (34.8-63.4)	51	72.5 (58.3-84.1)	52	40.4 (27.0-54.9)	154	53.9 (45.7-61.9)
Anti-HPV 31	238	69.7 (63.5-75.5)	75	100 (95.2-100)	55	58.2 (44.1-71.3)	52	69.2 (54.9-81.3)	56	41.1 (28.1-55.0)	163	55.8 (47.9-63.6)
Anti-HPV 33	238	77.7 (71.9-82.9)	76	100 (95.3-100)	55	69.1 (55.2-80.9)	53	75.5 (61.7-86.2)	54	57.4 (43.2-70.8)	162	67.3 (59.5-74.4)
Anti-HPV 45	243	65.0 (58.7-71.0)	82	100 (95.6-100)	54	44.4 (30.9-58.6)	52	65.4 (50.9-78.0)	55	32.7 (20.7-46.7)	161	47.2 (39.3-55.2)
Anti-HPV 52	248	78.2 (72.6-83.2)	90	100 (96.0-100)	53	67.9 (53.7-80.1)	52	73.1 (59.0-84.4)	53	56.6 (42.3-70.2)	158	65.8 (57.9-73.2)
Anti-HPV 58	235	81.7 (76.2-86.4)	74	100 (95.1-100)	53	73.6 (59.7-84.7)	54	79.6 (66.5-89.4)	54	66.7 (52.5-78.9)	161	73.3 (65.8-79.9)

The all type-specific naïve subjects with serology population included all participants that received all 3 vaccinations with the 9vHPV vaccine, were seronegative to the appropriate HPV type at Day 1, had serology results based on serum samples collected within pre-specified acceptable day ranges for ANSS population (21 to 105 days after dose 3). N= number of participants in each patient group that received at least one dose of the vaccine
n= number of patients contributing to the analysis
ANSS = All type-specific naïve subjects with serology, CI = Confidence interval, cLIA = competitive luminex immunoassay, GMT = Geometric mean titer, HPV= Human papilloma virus, mMU = milli-Merck unit, Tx = transplant

Supplementary table 2: Month 1 and month 7 geometric mean titers in the seropositive at baseline population

	All patients		HIV		Kidney Tx		Heart Tx		Lung Tx		All transplant	
	N=271		N=100		N=57		N=57		N=57		N=171	
cLIA assay Day 1	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**
Anti-HPV 6	46	98 (82-118)	29	101 (79-128)	5	74 (47-114)	9	115 (72-184)	3	80 (51-126)	17	95 (71-127)
Anti-HPV 11	22	69 (49-96)	15	71 (47-107)	2	58 (31-106)	4	76 (27-211)	1	38 (NA-NA)	7	64 (35-115)
Anti-HPV 16	36	103 (76-140)	28	85 (66-109)	3	649 (147-2870)	3	161 (47-550)	2	53 (42-68)	8	206 (78-542)
Anti-HPV 18	38	104 (81-134)	24	101 (82-124)	5	71 (58-86)	5	114 (55-235)	4	183 (26-1271)	14	110 (61-199)
Anti-HPV 31	26	58 (44-77)	21	54 (43-69)	1	34 (NA-NA)	4	95 (25-354)	0	NA	5	77 (26-231)
Anti-HPV 33	24	36 (30-45)	18	35 (28-44)	1	30 (NA-NA)	3	53 (19-151)	2	29 (27-30)	6	39 (23-67)
Anti-HPV 45	21	25 (20-31)	14	22 (18-26)	2	16 (15-17)	4	50 (28-91)	1	20 (NA-NA)	7	32 (19-54)
Anti-HPV 52	13	35 (24-52)	6	27 (21-34)	3	29 (18-45)	3	85 (23-304)	1	25 (NA-NA)	7	45 (23-88)
Anti-HPV 58	28	26 (20-33)	21	23 (18-28)	3	18 (16-19)	2	94 (69-128)	2	50 (23-108)	7	38 (21-69)
cLIA assay Month 7	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**	**n**	**GMT (95% CI), mMU/ml**
Anti-HPV 6	46	1236 (466-2316)	29	2140 (445-2210)	5	309 (76-455)	9	671 (326-1286)	3	387 (143-134)	17	485 (131-782)
Anti-HPV 11	22	808 (361-820)	15	1387 (362-822)	2	216 (200-171)	4	421 (199-458)	1	47 (NA-NA)	7	254 (90-77)
Anti-HPV 16	36	2988 (6567-6005)	28	3413 (7131-6521)	3	3382 (3393-611)	3	2152 (207-725)	2	632 (376-128)	8	1877 (2628-473)
Anti-HPV 18	38	787 (619-1255)	24	1332 (710-1440)	5	147 (88-40)	5	868 (468-698)	4	243 (11-9)	14	320 (76-34)
Anti-HPV 31	26	797 (630-295)	21	866 (708-331)	1	446 (NA-NA)	4	598 (192-807)	0	NA	5	564 (49-363)
Anti-HPV 33	24	448 (873-1715)	18	607 (889-1745)	1	150 (NA-NA)	3	211 (209-25)	2	156 (1-33)	6	180 (50-356)
Anti-HPV 45	21	208 (262-287)	14	258 (283-309)	2	31 (24-28)	4	325 (42-206)	1	79 (NA-NA)	7	135 (9-11)
Anti-HPV 52	13	246 (151-174)	6	381 (128-147)	3	214 (169-155)	3	159 (117-21)	1	99 (NA-NA)	7	169 (126-115)
Anti-HPV 58	28	336 (429-483)	21	458 (443-499)	3	46 (15-32)	2	410 (208-51)	2	213 (144-64)	7	134 (11-23)

The seropositive at baseline population included participants who were seropositive to the appropriate HPV type at Day 1, received all 3 vaccinations with the 9vHPV vaccine within pre-specified acceptable day ranges, had serology results based on serum samples collected within pre-specified acceptable day ranges and had no protocol deviations that could interfere with the subject's immune response to the 9vHPV vaccine as judged by the principal investigator.
N= number of participants in each patient group that received at least one dose of the vaccine
n= number of patients contributing to the analysis
ANSS = All type-specific Naïve subjects with serology, CI = Confidence interval, cLIA = competitive luminex immunoassay, GMT = Geometric mean titer, HPV= Human papilloma virus, mMU = milli-Merck unit, Tx = transplant

Supplementary table 3: predictors of geometric mean titers in HIV and SOT patients: PPI regardless of serostatus at day 1.

HIV group	**HPV6 GMT**	**HPV11 GMT**	**HPV16 GMT**	**HPV18 GMT**	**HPV31 GMT**	**HPV33 GMT**	**HPV45 GMT**	**HPV52 GMT**	**HPV58 GMT**
	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)
Seropositivity at baseline	**1.0 (0.5;1.4)*** **	**0.7 (0.2;1.2)***	**0.5 (0.2;0.9)****	**0.9 (0.5;1.3)*** **	**0.9 (0.4;1.3)*** **	**0.8 (0.4;1.3)*** **	**0.6 (0.1;1.1)***	-0.2 (-1.1;0.7)	**0.7 (0.3;1.1)****
Female sex (vs male)	0.5 (-0.2;1.2)	0.5 (-0.2;1.1)	-0.3 (-0.8;0.2)	-0.2 (-0.7;0.3)	-0.2 (-0.8;0.5)	0.1 (-0.5;0.6)	0.0 (-0.5;0.6)	0.3 (-0.4;1.0)	-0.1 (-0.7;0.4)
Age (years divided by 10)	-0.1 (-0.4;0.2)	-0.0 (-0.3;0.3)	-0.2 (-0.4;0.1)	-0.2 (-0.4;0.1)	-0.1 (-0.4;0.3)	0.0 (-0.2;0.3)	-0.3 (-0.5;0.0)°	0.1 (-0.2;0.4)	**-0.3 (-0.6;- 0.1)***
Origin									
Caucasian	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
African	**0.7 (0.1;1.3)***	0.6 (-0.0;1.2)°	**0.8 (0.4;1.3)****	**0.8 (0.3;1.3)****	**1.1 (0.5;1.7)*** **	**0.6 (0.1;1.2)***	**0.8 (0.3;1.4)****	**1.2 (0.6;1.9)*** **	**0.8 (0.2;1.3)****
Other [a]	0.0 (-0.7;0.8)	0.1 (-0.6;0.8)	0.2 (-0.4;0.8)	0.0 (-0.5;0.6)	-0.1 (-0.7;0.6)	0.5 (-0.1;1.1)	0.2 (-0.4;0.8)	0.0 (-0.7;0.8)	0.2 (-0.4;0.8)
BMI	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)°	0.0 (-0.0;0.1)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	-0.0 (-0.0;0.0)	-0.0 (-0.1;0.0)	-0.1 (-0.1;0.0)°	-0.0 (-0.1;0.0)
CD4+ T-cell count divided by 10	0.0 (-0.1;0.1)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	0.0 (-0.1;0.1)	-0.0 (-0.1;0.0)	**-0.1 (-0.2;0.0)****	0.0 (-0.1;0.1)	-0.0 (-0.1;0.0)
SOT patients	**HPV6 GMT**	**HPV11 GMT**	**HPV16 GMT**	**HPV18 GMT**	**HPV31 GMT**	**HPV33 GMT**	**HPV45 GMT**	**HPV52 GMT**	**HPV58 GMT**
	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)
Seropositivity at baseline	**1.4 (0.8;2.0)*** **	**1.1 (0.1;2.1)***	**1.9 (0.6;3.3)****	**1.2 (0.6;1.7)*** **	**2.3 (1.0;3.7)*** **	**1.4 (0.4;2.4)****	**1.6 (0.7;2.6)*** **	**1.2 (0.2;2.2)***	**1.6 (0.4;2.7)****
Female sex (vs male)	0.2 (-0.2;0.6)	0.3 (-0.1;0.8)	0.3 (-0.3;1.0)	0.2 (-0.2;0.5)	0.5 (-0.1;1.0)°	0.4 (-0.1;0.8)°	0.2 (-0.2;0.6)	0.4 (-0.1;0.9)°	**0.6 (0.1;1.1)***
Age (years divided by 10)	-0.0 (-0.2;0.2)	-0.1 (-0.4;0.1)	-0.1 (-0.5;0.2)	-0.1 (-0.2;0.1)	-0.1 (-0.4;0.1)	-0.1 (-0.3;0.1)	-0.0 (-0.2;0.2)	-0.1 (-0.4;0.1)	-0.1 (-0.3;0.2)
Transplant group									
Kidney Tx	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Heart TX	0.1 (-0.4;0.6)	0.2 (-0.4;0.8)	0.3 (-0.5;1.0)	**0.5 (0.1;0.9)***	0.4 (-0.3;1.0)	0.1 (-0.4;0.7)*	**0.5 (0.0;1.0)***	0.2 (-0.3;0.8)	0.3 (-0.3;0.9)
Lung TX	-0.0 (-0.6;0.5)	-0.3 (-0.9;0.4)	-0.4 (-1.3;0.5)	-0.3 (-0.8;0.2)	-0.4 (-1.2;0.3)	-0.0 (-0.6;0.6)	-0.3 (-0.9;0.3)	-0.3 (-0.9;0.4)	-0.1 (-0.8;0.6)
BMI	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	-0.0 (-0.1;0.0)	**-0.0 (-0.1;-0.0)***	-0.0 (-0.1;0.0)°	-0.0 (-0.1;0.0)
Years since transplantation	-0.0 (-0.0;0.0)	-0.0 (-0.0;0.0)	-0.0 (-0.1;0.1)	0.0 (-0.0;0.0)	0.0 (-0.0;0.0)	0.0 (-0.0;0.0)	-0.0 (-0.0;0.0)	0.0 (-0.0;0.0)	0.0 (-0.0;0.1)
Immunosuppression at baseline, n	**-0.8 (-1.3;-0.2)****	-0.4 (-1.1;0.2)	**-1.0 (-1.8;-0.1)***	-0.1 (-0.5;0.4)	-0.5 (-1.2;0.2)	**-0.6 (-1.2;-0.1)***	-0.4 (-0.9;0.2)	-0.4 (-1.0;0.2)	**-0.8 (-1.5;-0.1)***
Mycophenolate mofetil	**-0.9 (-1.3;-0.4)*** **	**-1.0 (-1.5;-0.5)*** **	**-1.1 (-1.8;-0.4)****	**-0.7 (-1.1;-0.4)*** **	**-1.0 (-1.6;-0.5)*** **	**-0.7 (-1.2;-0.2)****	**-0.7 (-1.2;-0.2)****	**-0.7 (-1.2;-0.2)****	**-0.8 (-1.4;-0.2)****
Tacrolimus	**-0.8 (-1.5;-0.1)***	-0.7 (-1.4;0.1)	**-1.1 (-2.2;-0.1)***	**-0.7 (-1.2;-0.1)***	**-0.9 (-1.8;-0.1)***	-0.5 (-1.2;0.2)	-0.5 (-1.2;0.2)	-0.5 (-1.3;0.3)	**-0.9 (-1.7;0.0)***

This analysis included all participants regardless of serostatus at day 1, who received all 3 vaccinations with the 9vHPV vaccine within pre-specified acceptable day ranges, had serology results based on serum samples collected within pre-specified acceptable day ranges and had no protocol deviations that could interfere with the subject's immune response to the 9vHPV vaccine as judged by the principal investigator.
[a] Other included Asian and Latin-American origin. b= regression coefficient, TX = transplant, BMI= Body Mass Index, °p< 0.1, *p<0.05, **p<0.01, ***p<0.001.

CHAPTER 4: Vaccination coverage of recommended vaccines and determinants of vaccination in at-risk groups

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Abstract

Upon exposure to vaccine-preventable diseases, certain individuals are at increased risk for complications due to pre-existing diseases, age or immunosuppressive treatment. Vaccination against influenza, pneumococcal disease and hepatitis B (for some groups) is advised in addition to standard vaccination against diphtheria, tetanus and pertussis. We estimated the vaccination coverage and determinants of recommended vaccinations in patients with diabetes mellitus type 1 (n= 173) and type 2 (n=177), chronic kidney disease (CKD) (n=138), heart failure (n=200), chronic obstructive pulmonary disease (COPD) (n=187), HIV (n=201) or solid organ transplantation (SOT) (n=255) in a monocentric study. Vaccination data were retrieved from documents provided by patients and general practitioners, and from the Flemish vaccination register. Less than 10% had received all recommended vaccines. Overall, 29% of subjects were vaccinated against diphtheria-tetanus, 10% against pertussis, 44% against influenza, 32% against pneumococcal disease and 24% of HIV patients and 31% of CKD patients against hepatitis B. Age was positively associated with vaccination against influenza (OR:2.0, $p<0.01$) and pneumococcal disease (OR:2.6, $p<0.001$). Patients with COPD, HIV and SOT were more likely to be vaccinated against influenza (OR:2.8, $p<0.001$, OR:1.8, $p<0.05$; OR:2.0, $p<0.001$, respectively) and pneumococcal disease (OR:2.9, $p<0.001$, OR:25.0, $p<0.001$; OR:2.6, $p<0.001$, respectively) than patients with heart failure. Reason for non-vaccination were concerns about effectiveness, necessity and side effects of influenza vaccines, and not being aware of the recommendation for pneumococcal disease. Initiatives to monitor the vaccination status of vulnerable patients are needed, which is why we advocate systematic vaccination registration and frequent communication about vaccination.

Keywords: recommended vaccines, vaccination coverage, determinants, at-risk groups

Introduction

The number of people with immunosuppressive conditions and chronic diseases is growing [1,2]. Due to the nature of their condition, immunosuppressive treatment or their age, these individuals are at increased risk of developing complications upon exposure to infectious pathogens, including those against which they can be vaccinated. For example, patients with diabetes mellitus (DM) are up to 25 times more likely to develop pneumonia and 6 times more likely to be hospitalized upon influenza infection compared to the general population [3]. Additionally, they have a higher risk of acquiring nosocomial infection as they frequently visit hospitals for disease follow-up. In patients with a chronic disease, infection can also lead to a deterioration of their condition. For example, in patients with chronic obstructive pulmonary disease (COPD), certain infectious agents such as influenza, *Bordetella pertussis* and *Streptococcus pneumoniae* cause respiratory disease, which may lead to COPD exacerbation [4]. In DM patients, infection with influenza may cause metabolic dysregulation and increase blood glucose to precariously high levels [5]. Vaccination is the best available measure to prevent infection and to decrease morbidity and mortality. For example, influenza vaccination reduces all-cause hospitalization and hospitalization due to influenza or pneumonia in diabetes patients, and all-cause mortality and cardiovascular mortality in patients with heart failure [6,7]. Hence, it is highly recommended that at-risk patients follow the standard vaccination schedule, with some additions or minor adaptations specific for their condition. It is recommended for all adults to receive a ten-yearly booster of a tetanus and diphtheria vaccine after a primary schedule of at least 3 doses [8]. In line with the recommendations of the Advisory Committee on Immunization practices (ACIP) in the United States, the Belgian National Immunization Technical Advisory Groups (NITAG) advises to use for this at least once a tetanus, diphtheria and acellular pertussis (Tdap) vaccine [9,10]. For seasonal influenza, the NITAG only recommends annual vaccination for people aged 65 years and older, and for all patients with chronic diseases [10,11]. This is in line with recommendations in other countries, like the United Kingdom, but narrower than the ACIP recommendation of annual vaccination for all adults [9,10]. Pneumococcal vaccination is also recommended for this target group by most public health authorities [9,10,12]. The Belgian NITAG recommends using the 13-valent conjugate pneumococcal vaccine (PCV13), followed by a dose of the 23-valent polysaccharide pneumococcal vaccine (PPSV23) with an interval of at least 8 weeks, and subsequently a PPSV23 booster every five years in immunocompromised patients since 2013 [10]. Additionally, some vaccines are recommended for particular at-risk groups. In accordance with the World Health Organization (WHO) and ACIP, Belgian recommendations also include vaccination against hepatitis B for people with an increased risk of exposure to infected blood, such as patients with HIV, DM, CKD and solid organ transplantation (SOT) candidates [9,10,13]. Furthermore, the Belgian NITAG recommends vaccination with live attenuated vaccines against measles, and mumps, rubella and varicella, but only for non-immune HIV patients with a CD4-count of at least 200 cells/µl and SOT candidates. Live vaccines are, however, contra-indicated in immunocompromised patients. Finally, meningococcal vaccination is recommended for immunocompromised patients with an increased personal or epidemiological risk, and human papilloma virus vaccination has been recommended for adult immunocompromised patients since 2017 [10].

Despite these recommendations, few countries monitor or report vaccination coverage in at-risk groups [8]. The European Centre for Disease Prevention and Control (ECDC) reported that less than 25% of the member states recorded influenza vaccination coverage in such target groups, and existing data generally indicate low uptake [14,15-19]. During a vaccination coverage survey in the general population of children and adolescents in Flanders, illness was frequently given as reason for non-vaccination [20]. In 2013, self-reported vaccination coverage was 50% for influenza in the past year and 8% for pneumococcal disease in the past 5 years [21]. Self-reported vaccination is, however, not deemed reliable because an overestimation of the true vaccination coverage may occur due to participation, recall and social desirability bias and underestimation of the time interval since the last vaccination [22]. So far, studies on documented uptake of recommended vaccines in pediatric or adult at-risk groups have not yet been performed. We assessed vaccination status and determinants of Tdap, seasonal influenza, pneumococcal and hepatitis B vaccinations in adult patients with DM, CKD, COPD, heart failure, HIV and SOT.

Methods

Study Procedure and Population

This is a monocentric cross-sectional survey in adult at-risk patients at the university hospitals of Leuven, which is the largest tertiary hospital in Belgium. It counts almost 10 000 employees, has 1764 beds and accounts for more than 700 000 outpatient visits annually [23]. Patients were approached in the outpatient clinics during consecutive six-month periods (one per patient group) between September 2014 and December 2018. All subjects lived in the Flemish region of Belgium, were at least 18 years of age and had either diabetes mellitus (DM) type 1, DM type 2, heart failure, COPD, CKD, HIV or a history of solid organ (heart or lung) transplantation. The questionnaire was based on a list of questions used in several vaccination coverage studies in children and adolescents between 2005 and 2012, but adapted to the current adult patient population [24]. The questionnaire was tested for clarity and feasibility before the start of data collection. As an example, an English translation of the questionnaire aimed at patients with SOT is available as supplementary data. The survey was taken as a structured interview based on the questionnaire and contained questions on vaccination status, reasons for non-vaccination, socio-economic and socio-demographic characteristics and disease characteristics. Reasons for non-vaccination were only surveyed in patients who had documented vaccination data (e.g. vaccination booklet) available at the time of the survey and/or who were aware of not being vaccinated for at least one recommended vaccine (n=367). Disease severity was determined with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) for COPD, Kidney Disease Improving Global Outcomes (KDIGO) for CKD, and the New York Heart Classification (NYHA) for heart failure [25-27]. Vaccination records were required for vaccination rate calculation. They were retrieved from documents provided by patients, from the general practitioner's medical records, and from Vaccinnet, the Flemish vaccination register. Vaccination data for hepatitis B were collected for patients with HIV and CKD only. Hepatitis B vaccination data were not collected from patients with DM because the vaccine is not systematically offered in our hospitals, since the risk of infection due to the exchange of needles from blood glucose measurement devices is considered limited. Signed informed consent was obtained from

all participants and the study was approved by the Ethics Committee Research UZ/KU Leuven of Leuven, Belgium (S56765).

Definitions of correct vaccination

Correct vaccination against diphtheria and tetanus was defined as a complete course of primary vaccination with 3 doses of a diphtheria and tetanus containing vaccine, and subsequent booster vaccinations every 10 years. In the present study we estimated the coverage of booster vaccination only (i.e. vaccination in the previous 10 years), assuming the basic schedule is complete. Correct vaccination for seasonal influenza implied having been vaccinated during the last vaccination campaign before the survey. Pneumococcal vaccination was surveyed for the 5 years preceding the survey and required having been vaccinated at least once. Correct hepatitis B vaccination equaled i) 4 doses or ii) 3 doses with an interval of at least four weeks between dose 1 and 2, eight weeks between dose 2 and 3 and 16 weeks between dose 1 and 3. Doses were considered invalid if the vaccines were administered more than 5 days before these recommended intervals. Pertussis vaccination was considered correct if the patient had received at least one dose of a pertussis-containing vaccine. Since adult pertussis vaccination has only been recommended in Belgium since 2013, no time restriction for correct vaccination was needed in the present analysis.

Statistical Analysis

We calculated a sample size of 250 patients per disease group to estimate an expected vaccination coverage of 70% with a confidence interval of approximately ± 6% [21]. This sample size also allowed to detect differences between disease groups of approximately 10% with a power of 80%. Vaccination coverage rates of recommended vaccines are shown with binomial 95% confidence intervals. Multivariate logistic regression with backwards selection was used to analyze determinants of vaccination coverage for each vaccine independently. A test probability of 5% was considered statistically significant. All data were analyzed with R. version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, 2013).

Results

Patient characteristics

The response rate was about 90% in all patient groups, except for COPD (49%), because other studies were running simultaneously, and patients preferred to participate in one study at a time. Other reasons for refusal were lack of interest and time, or not feeling well. In total 1331 patients were included, with either DM type 1 (n=173), DM type 2 (n=177), CKD (n=138), COPD (n=187) heart failure (n=200), HIV (n=201) or SOT (n=255). Table 1 shows patient characteristics. The majority of participants were male (62.7%) and above the age of 40 (86.7%). All CKD patients had a severe disease status (KDIGO classification ≥4). In total, 57.8% of the COPD patients and 41.0% of the heart failure patients had a severe disease state (GOLD stage C or D and NYHC class 3 or 4, respectively). In the SOT group, 128 were lung transplant patients and 127 heart transplant patients. Of the HIV patients, 46.9% were men who have sex with men (MSM) and 98% had a CD4+ count of ≥200 cells/mm².

Table 1: Patient characteristics

	All patients (n=1331)	DM type 1 (N =173)	DM type 2 (N =177)	CKD (N=138)	COPD[b] (N=187)	Heart failure[c] (N=200)	HIV (n=201)	SOT (n=255)
Personal data	%	%	%	%	%	%	%	%
Median age, years (range)	62 (18-94)	44 (18-83)	67 (31-91)	73 (21-91)	65 (29-94)	71.5 (32-91)	46(18-75)	60 (19-87)
Age								
< 40 years	13.3	38.7	1.7	3.6	0.5	1.0	30.9	14.5
40-64 years	43.8	46.8	40.1	19.6	45.5	27.5	64.2	52.9
≥ 65 years	42.9	14.5	58.2	76.8	54.0	71.5	5.0	32.6
Female gender	33.7	46.8	35.6	34.8	32.6	30.5	26.4	32.2
Origin								
Belgian	85.5	86.7	91.5	96.4	91.4	92.5	58.2	86.3
European	8.1	8.1	6.2	3.6	7.5	4.5	10.0	13.7
Non-European	6.4	5.2	2.3	0.0	1.1	3.0	31.8	0.0
Educational degree[a] (years of study)								
Lower education (<12 years)	36.7	11.6	52.5	50.7	42.8	50.0	22.4	31.4
Secondary education (12 years)	33.6	42.8	25.4	29.7	35.3	28.0	38.3	34.5
Higher education (>12 years)	28.8	45.7	22.0	18.1	21.9	19.5	38.3	32.5
Unknown education	1.0	0.0	0.0	1.4	0.0	2.5	1.0	1.6
Employed (full + part time)	67.8	80.9	75.7	69.6	66.3	65.0	70.6	53.3
Net monthly family income								
<1500 euro	23.1	13.3	22.6	23.2	27.4	30.0	26.4	18.8
1500-3000 euro	46.8	49.7	59.9	42.8	46.2	44.0	36.3	49.0
>3000 euro	19.3	36.4	13.0	10.9	8.1	14.0	32.3	18.8
Unknown income	10.8	0.6	4.5	23.2	18.3	12.0	5.0	13.3
Civil status: married/cohabitation	69.0	71.7	71.2	70.3	70.6	66.5	57.2	74.9
Physical activity								
Never	22.5	25.4	32.8	42.0	13.4	28.5	16.9	9.4
Occasionally (≤2 times/week)	15.6	30.6	24.9	18.8	2.1	8.0	16.4	12.2
Frequently (>3 times/week)	62.0	43.9	42.4	39.1	84.5	63.5	66.7	78.4
Smoking								
No smoking	38.7	55.5	40.7	44.2	4.3	35.5	48.8	42.7
Smoker	15.0	17.3	17.5	12.3	17.1	9.5	28.4	5.1
Ex-smoker	46.4	27.2	41.8	43.5	78.6	55.0	22.9	52.2
Alcohol use								
No	50.7	48.6	59.9	58.0	57.8	45.5	33.8	54.1
Occasionally (1-7 glasses/week)	37.0	35.8	27.7	31.2	22.5	45.0	51.2	40.4
Frequently (>7 glasses/week)	12.3	15.6	12.4	10.9	19.8	9.5	14.9	5.5
Disease data								
Comorbidity	44.9	9.2	47.5	64.5	73.3	47.0	31.3	44.7
Years since diagnosis/ transplantation (median (range))	8 (0-64)	17.5 (0-59)	11 (0-64)	4.5 (1-57)	6 (0-39)	6 (0-60)	9 (0-30)	7 (1-29)

[a] Educational degree: Lower Education= no secondary school diploma, Secondary education = secondary school diploma achieved, Higher education= university of university college diploma achieved.

[b] Patients were classified in categories of disease severity according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) GOLD stages: 19.3 % had GOLD stage A, 23.0% GOLD stage B, 10.2% GOLD stage C and 47.6% GOLD stage D. The severity of symptoms is measured with the Modified Medical Research Council Dyspnea Scale (mMRC) and the COPD Assessment Test (CAT). Patients with GOLD A and B are at low risk (0-1 exacerbation per year, not requiring hospitalization), GOLD C and D are high risk patients (≥2 exacerbations per year, or one or more requiring hospitalization). GOLD A and C have few symptoms (mMRC 0-1 or CAT <10), GOLD B and D have more symptoms (mMRC≥ 2 or CAT≥ 10) [24].

[c] Patients were classified in categories of disease severity according to New York Heart Classification (NYHA): 25.5% had class I (no limitation in ordinary physical activity), 33.0% class II (Mild symptoms and slight limitation during ordinary activity and comfortable at rest), 36.0% had class III (Marked limitation in activity due to symptoms, even during less-than-ordinary activity and comfortable only at rest) and 5.0% had class IV (severe limitations and experiences symptoms even while at rest) [25].

COPD: chronic obstructive pulmonary disease, CKD: chronic kidney disease, DM: Diabetes mellitus, SOT: Solid organ transplantation.

Vaccination coverage in adults with chronic diseases

About 10% of the patients had a vaccination document available at the time of the survey and an additional 3% mailed a copy afterwards. The general practitioner's response rate varied from 50 to 75 %, depending on the disease group. Documented proof of at least one of the studied vaccines could only be found for 68.7% of the patients. In total, only 9.8% of the patients was correctly vaccinated with the recommended vaccines (excluding pertussis and hepatitis B). In all groups, coverage rates were relatively low for all recommended vaccines (table 2). About 30% were vaccinated against diphtheria-tetanus, 10% against pertussis, 44% against influenza and 32% against pneumococcal disease. In total, 25% of HIV patients and 30% of CKD patients were vaccinated against hepatitis B. Another 8% of CKD patients and 3% of HIV patients were possibly still on a hepatitis B vaccination trajectory as the last vaccine of their incomplete schedule was administered less than one year before the survey. Among the different groups, COPD patients had the highest coverage rates for diphtheria/tetanus and influenza; and HIV patients for pneumococcal disease. The self-reported vaccination coverage rate was 45.2% for diphtheria/tetanus, 35.6% for pneumococcus and 81.2% for influenza.

Table 2: Documented vaccination coverage in adult risk patients

n=1331	**Diphtheria-Tetanus**		**Pertussis**		**Influenza**		**Pneumococcus**		**Hepatitis B**	
	n	**% (95%CI)**	**n**	**% (95%CI)**	**n**	**% (95%CI)**	**n**	**% (95%CI)**	**n**	**% (95%CI)**
All patients (n=1331)	387	29.1 (26.7-31.6)	136	10.2 (8.7-12.0)	584	43.9 (41.2-46.6)	429	32.2 (29.7-34.8)		NA
DM type 1 (n=173)	45	26.0 (19.8-33.3)	22	12.7 (8.3-18.8)	39	22.5 (16.7-29.6)	7	4.0 (1.8-8.5)		NA
DM type 2 (n=177)	54	30.5 (23.9-37.9)	29	16.4 (11.4-22.9)	85	48.0 (40.5-55.6)	43	24.3 (18.3-31.4)		NA
CKD (n=138)	33	23.9 (17.2-32.1	7	5.1 (2.2-10.6)	39	28.3 (21.1-36.7)	32	23.2 (16.6-31.3)	43	31.2 (23.7-39.7)
COPD (n=187)	65	34.8 (28.1-42.1)	23	12.3 (8.1-18.1)	121	64.7 (57.4-71.4)	75	40.1 (33.1-47.5)		NA
Heart failure (n=200)	58	29.0 (22.9-35.9)	17	8.5 (5.2-13.5)	77	38.5 (31.8-45.7)	40	20.0 (14.8-26.4)		NA
HIV (n=201)	61	30.3 (24.2-37.3)	7	3.5 (1.5-7.3)	88	43.8 (36.9-50.9)	146	72.6 (65.8-78.6)	49	24.4 (18.7-31.0)
SOT (n=255)	71	27.7 (22.5-33.8)	31	12.2 (8.5-17.0)	135	52.9 (46.6-59.2)	86	33.7 (28.0-39.9)		NA

NA: not available, CI: confidence interval, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, DM: Diabetes mellitus, SOT: solid organ transplantation

Determinants of vaccination coverage in adults with chronic diseases

Factors associated with immunization are shown in table 3. For diphtheria-tetanus vaccination, no significant determinant was found. Against pertussis, patients with DM type 2 (OR: 2.3 p<0.01) were proportionally better vaccinated than patients with heart failure. Moreover, those who were occasionally physically active (≤2 times/week) were less likely to be vaccinated than those who were never physically active (OR: 0.5, p<0.05). The influenza vaccination coverage was higher in patients with DM type 2 (OR: 1.6, p < 0.05), COPD (OR: 2.8, p < 0.001), HIV (OR: 1.8, p<0.001) and SOT (OR: 2.0, p<0.001) compared to heart failure patients. In addition, a significant increase in influenza vaccination was observed in the age groups 40-64 years (OR: 1.6, p<0.05) and ≥ 65 years (OR: 2.0, p<0.01) compared to the younger age groups. Lastly, patients who were frequently physically active (≥3 times/week) were more likely to be vaccinated than those who were never physically active (OR: 1.4, p<0.05). Pneumococcal vaccination rates were higher in COPD (OR: 2.9, p<0.001), SOT (OR: 25.0, p<0.001) and HIV patients (OR: 2.6, p<0.001), but lower in CKD patients (OR: 0.3, p<0.001) compared to heart failure patients. Patients in older age groups were better vaccinated (OR: 2.6, p<0.001), and ex-smokers as well (OR: 1.5 vs. non-smokers, p<0.05). Against hepatitis B, patients with HIV were less well vaccinated than those with CKD (OR: 0.5, p<0.05).

In addition to these demographic factors, univariate analysis showed that patients who received information on specific vaccines were better vaccinated against pneumococcus (OR: 4.8, p<0.001).

Table 3: Determinants of recommended vaccinations in adult patient groups: Multivariate logistic regression

n=1331	Diphtheria-Tetanus	Pertussis	Influenza	Pneumococcus	Hepatitis B
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Age					
< 40 years			Reference	Reference	
40-64 years			**1.6 (1.1-2.4)***	1.6 (1.0-2.7)°	
≥ 65 years			**2.0 (1.3-3.2)****	**2.6 (1.5-4.6)*****	
Female gender				1.3 (1.0-1.8)	0.6 (0.3-1.1)
Disease group					
DM type 1		1.8 (0.9-3.6)°	0.7 (0.4-1.1)°	**0.3 (0.1-0.6)****	NA
DM type 2		**2.3 (1.2-4.5)****	**1.6 (1.1-2.5)***	1.4 (0.8-2.3)	NA
CKD		0.6 (0.2-1.5)	0.7 (0.4-1.1)	1.3 (0.8-2.2)	Reference
COPD		1.5 (0.8-2.9)	**2.8 (1.8-4.3)*****	**2.9 (1.8-4.7)*****	NA
Heart failure		Reference	Reference	Reference	NA
HIV		0.4 (0.2-1.0)°	**1.8 (1.1-2.8)***	**25.0 (13.9-46.3)*****	**0.5 (0.3-0.9)***
SOT		1.5 (0.8-2.9)	**2.0 (1.3-3.0)*****	**2.6 (1.6-4.1)*****	NA
Origin					
Belgian				Reference	
European				0.7 (0.4-1.2)	
Non-European				0.6 (0.3-1.0)°	
Educational degree[a] (years of study)					
Lower education (<12 years)	0.8 (0.6-1.1)				
Secondary education (12 years)	Reference				
Higher education (>12 years)	1.2 (0.9-1.6)				
Unknown	0.7 (0.2-2.3)				
Employment					
Full-time				Reference	
Part-time				0.7 (0.4-1.2)	
Not working				1.3 (0.9-1.8)	
Net monthly family income					
<1500 euro				0.8 (0.6-1.1)	1.2 (0.6-2.4)
1500-3000 euro				Reference	Reference
>3000 euro				1.2 (0.8-1.7)	**2.7 (1.4-5.3)****
Unknown income				**0.5 (0.3-0.8)****	1.0 (0.4-2.2)
Physical activity					
Never		Reference	Reference		
Occasionally (≤2 times/week)		**0.5 (0.2-1.0)***	1.4 (1.0-2.1)°		
Frequently (>3 times/week)		1.0 (0.7-1.6)	**1.4 (1.1-1.9)***		
Smoking					
No smoking			Reference	Reference	
Smoker			0.9 (0.6-1.2)	1.0 (0.7-1.6)	
Ex-smoker			1.2 (0.9-1.6)	**1.5 (1.1-2.1)***	

Multivariate logistic regression with backwards selection

[a] Education: Lower Education= no secondary school diploma, Secondary education = secondary school diploma achieved, higher education= university of university college diploma achieved. CI: confidence interval, CKD: chronic kidney disease COPD: chronic obstructive pulmonary disease, DM: diabetes mellitus, SOT: solid organ transplantation, ° p <0.1; * p <0.05; ** p <0.01; *** p <0.001

Reasons for non-vaccination and information provided about vaccination

Table 4 lists the reasons for non-vaccination with a particular vaccine. For diphtheria-tetanus, frequently given reasons were not being informed about the recommendation (38%) and having forgotten it (29%). For influenza, 41% stated that they planned to receive the vaccine. Other reasons were concerns about the vaccine's safety (13%), necessity (6%) and effectiveness (6%), or opposition against influenza vaccination (9%). For pneumococcal vaccination, 89% was not aware of the recommendation.

For influenza, 71% of the patients stated that they had received information concerning vaccination against the disease. Of those patients, 60% received the information from their general practitioner and 30% from a specialist. For pneumococcal vaccination, 29% of the patients received information about the vaccine. Of those patients, 48% was informed by their general practitioner and 47% by a specialist. Other sources of information for influenza and pneumococcal vaccination were occupational health professionals, family and friends.

Table 4: reasons for non-vaccination

N= 367	**Diphtheria-tetanus (n=86) n(%)**	**Influenza (n=157) n(%)**	**Pneumococcus (n=138) n(%)**
Concerns and doubts			
Concerns about safety	1 (1.2)	20 (12.7)	1 (0.1)
Doubts about necessity of vaccination	8 (9.3)	10 (6.4)	2 (1.4)
Doubts about effectiveness of vaccination	-	10 (6.4)	-
Opposition to vaccination	-	14 (8.9)	5 (3.6)
Afraid of needle	-	1 (0.6)	-
Does already take a lot of medication	-	1 (0.6)	-
Information			
Not aware of the recommendation	33 (38,4)	4 (2.5)	111 (80.4)
Discouraged by physician	-	4 (2.5)	-
Assumed not to be necessary since absence of injuries	4 (4.6)	-	-
Practical reasons			
Having forgotten to get the vaccine	25 (29.1)	1 (0.6)	3 (2.2)
I have not received vaccine yet, but will get it in the future	-	64 (40.7)	2 (1.4)
Vaccine is too expensive	-	-	1 (0.7)
Not given due to medical condition/treatment	-	3 (1.9)	-
Lack of time	-	1 (0.6)	-
No reason	12 (14.0)	23 (14.6)	13 (9.4)

Discussion

Less than 10% of the patients were vaccinated against diphtheria-tetanus, influenza and pneumococcal disease. Overall, 29% of the subjects were vaccinated against diphtheria-tetanus, 10% against pertussis, 44% against influenza, 32% against pneumococcal disease and 24% of patients with HIV and 31% of patients with CKD were vaccinated against hepatitis B.

For influenza, the vaccination coverage is far below the WHO/EU target of 75% for at-risk groups [28,29]. Similarly, the WHO European region reported coverage rates of mostly below 40% for people with chronic illnesses in 14 other European countries [15]. For pneumococcal vaccination, other studies also reported low coverage rates ranging from 7% in Italy to 50% in immunocompromised patients in the United states and 60% in high-risk groups in Catalonia (Spain) [17-19,30]. A possible reason for low coverage rates is that patients at risk are closely monitored by a specialist and therefore less often consult a general practitioner, which is the preferred vaccinator in Belgium. Specialists often do not approach patients about vaccination as this is considered the general practitioner's task [1]. A vaccination recommendation by a specialist could thus have a substantial impact on the vaccination rate.

In accordance with other studies, we observed that people over the age of 65 are better vaccinated against influenza and pneumococcal disease [16,18,30-32]. Similarly, an Irish study found coverage rates of 28% against influenza and 16% against pneumococcus in adults at risk below 65 years of age and 60% against influenza and 36% against pneumococcus in adults above the age of 65 [18]. It has been suggested that most countries are more devoted to vaccinating older rather than younger at-risk groups [16]. Nevertheless, younger patients with chronic diseases are also at increased risk of complications, and neither recommendations nor uptake of vaccination should be different. Coverage for pneumococcal vaccination in diabetes patients might be low because they are not specifically mentioned as risk-group in the Belgian recommendations. However, it was generally recognized that this was a mistake as there is sufficient evidence that diabetes patients are at increased risk of pneumococcal disease [33,34]. Furthermore, SOT patients, who are the most immunocompromised, and COPD patients were more likely to be vaccinated against pneumococcal disease and influenza compared to patients with heart failure. Likewise, other studies report higher coverage rates in patients who are immunocompromised or suffer from a lung disease [17,18, 30]. Possibly, more attention is paid to their influenza and pneumococcal vaccination status due to the risk of COPD exacerbation. Since influenza has been associated with worsening of pre-existing heart disease, one might also expect a higher coverage in patients with heart failure [35], but in contrast with some other studies, we did not observe this trend [17,19, 30].

Given the low vaccination coverage rates for all recommended vaccines, it is clear that more effort is needed to monitor the vaccination status of patients at risk more closely. This starts with systematic registration and documentation of vaccination. We only found documented proof of any vaccination in less than 70% of the patients, but self-reported vaccination rates were higher. Although we cannot exclude recall bias, we attribute this difference mainly to issues with recordkeeping. Access to a central vaccination register is essential for both patients and healthcare providers to keep an overview of the

vaccination status [36]. Patients are often being followed up by different physicians (specialist, occupational health physicians, general practitioners) and vaccination records may become fragmented or lost. There is a central vaccine register (Vaccinnet) in Flanders (Belgium) which could resolve this issue, but it is not yet being used systemically for vaccines that are not available free of charge in Flanders. Therefore, registration of influenza, pneumococcal and (adult) hepatitis B vaccines is incomplete or missing, even for at-risk groups. Only the Tdap vaccine is provided free of charge to all.

In addition, physicians should address reasons for non-vaccination. In terms of influenza vaccination, concerns about effectiveness and side effects were important drivers for a lower uptake. Giese *et al*. reported not deeming vaccination necessary as the main reason for non-vaccination against influenza in adult risk patients below 65 years of age [18]. The most prevalent reason for non-vaccination against pneumococcal disease was not being aware of the recommendation. We found that less than 30% of all patients claimed to have received information about the pneumococcal vaccine. Similarly, according to a large European survey in people above 65 years, 54% stated that their physician had not recommended pneumococcal vaccination [37]. As we, and others, observed a strong positive association between the recommendation of a particular vaccine by a physician and the coverage rate of this vaccine, we urge all physicians to discuss this with their patients [18,31]. Furthermore, as some patients claimed to have forgotten the vaccination, a timely reminder by their physician would be beneficial.

Based on these findings, we advocate well-organized multi-intervention vaccination campaigns in which improving recordkeeping of administered vaccines and vaccination recommendations to patients by healthcare professionals are key components. Other studies showed a significant increase in vaccination uptake as a result from such an approach [38,39]. The guide to Tailoring Immunization Programs (TIP) from the World Health Organization could be used to tailor interventions to lower local barriers to vaccination [40]. Moreover, specific education in vaccinology for medical doctors and nurses should increase specialists' awareness of the issue and encourage them to recommend vaccines. Currently, vaccine education is limited in the training of physicians and nurses.

A strength of this first survey is that it assesses vaccination coverage of recommended vaccines in a large and diverse group of at-risk patients. Vaccination data in at-risk groups are scarce and often not monitored. Available studies are often limited to a particular vaccine in a certain at-risk group. However, there are some limitations to this survey as well. Firstly, not all recommended vaccines for adult at-risk patients are covered in the study. This includes vaccination against meningococcal disease, varicella, measles, mumps and rubella, which are only recommended in particular subgroups of our patient groups. Secondly, the external validity of the study may be limited as only patients attending a tertiary care hospital were surveyed. Studies on documented vaccination coverage of community at-risk patients are needed to assess follow-up of vaccination recommendation at population level. Nevertheless, we believe that surveys in such hospitals are important as they are responsible for teaching and training of health care workers and should set an example for other care settings. Thirdly, there is a possibility of selection bias, particularly in the HIV group. Patients who were not therapy compliant, recently diagnosed or changing therapy during the recruitment period were not approached

as resolving those issues was considered more important than study participation. Another drawback is the frequent lack of documentation. Since we only considered documented vaccination, our estimates are a lower boundary, which may well be an underestimation of the true coverage rates. We chose not to include self-reported data because studies have shown that recall bias by patients is large and such vaccination coverage rates are often overestimated [22,41]. Finally, we did not achieve the sample size of 250 patients in all groups due to time and logistical limitations. This resulted in an increase of the 95% CI width of coverage rates of up to one third in the smallest group (i.e. CKD, n=138).

We conclude that vaccination coverage of recommended vaccines in clinical risk groups is beneath the desired level. Efforts should be made to closely monitor the vaccination status of vulnerable groups. There is need for systematic vaccination registration, communication about vaccination by physicians and vaccination campaigns tailored to the at-risk groups.

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CHAPTER 5: Seroprevalence of antibodies against diphtheria, tetanus and pertussis in adult at-risk patients

Manuscript in preparation

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Abstract

Background

Patients with chronic diseases are at increased risk for complications following exposure to vaccine-preventable diseases. We assessed seroprevalence of antibodies against diphtheria, tetanus and pertussis to evaluate whether current vaccination programs in Belgium are adequate.

Methods

Antibody titers were assessed with a bead-based multiplex assay in serum of adults with diabetes mellitus type 1 (DM1) (n=172), DM2 (n=77), chronic kidney disease (n=130), chronic obstructive pulmonary disease (COPD) (n=170), heart failure (n=77), HIV (n=196) or a solid organ transplant (SOT) (n=230). Factors associated with seroprevalence were analyzed with multiple logistic regression.

Results

Seroprotective titers were reached in 29% for diphtheria (≥0,1IU/ml), in 83% for tetanus (≥0,1IU/ml), and seropositive titers in 22% for pertussis (≥5IU/ml). Seroprotection rates were higher ($p<0.001$) when vaccinated <10 years ago. Furthermore, diphtheria seroprotection decreased with age ($p<0.001$) and was less attained in COPD and SOT patients compared to DM1 patients ($p<0.01$). Tetanus seroprotection was less reached in women ($p<0.001$) and older age groups ($p<0.001$). For pertussis, women had more often a titer suggestive of a recent infection or vaccination (≥100IU/ml, $p<0.01$).

Conclusion

Except for tetanus, the vast majority of at-risk patients remains susceptible to vaccine-preventable diseases such as diphtheria and pertussis.

Keywords: seroprevalence, seroprotection, adults with chronic disease, vaccination, immunity, diphtheria, tetanus, pertussis

Introduction

Patients with a chronic condition are often at increased risk for complications upon exposure to infectious diseases. This can be explained by their clinical condition, age or immunosuppressive treatment. Although the exact contribution of underlying conditions to infectious disease outcome is not completely elucidated, it is known that patients hospitalized with severe pertussis often have co-morbidities [1]. Almost 30% of adult patients hospitalized with severe pertussis have chronic pulmonary obstructive disease (COPD), and patients with COPD have a 2.5 fold increased risk of being hospitalized due to pertussis [1,2]. Moreover, it has been suggested that pathogens such as *Bordetella pertussis* lead to exacerbation of the underlying condition. For example, a study in the United States reported that among adult patients hospitalized with severe pertussis infection, a large proportion were COPD patients who were admitted due to COPD exacerbation [1]. Hence, patients with a chronic disease might end up in a vicious circle where the condition promotes infection and infection worsens the condition [3]. To avoid this, it is imperative that they get vaccinated according to recommendations. The Belgian national immunization technical advisory group (NITAG) advises an adult booster dose with a tetanus and diphtheria containing vaccine every 10 years, which has to include at least once an acellular pertussis component (Tdap vaccine). Whereas childhood vaccination programs mostly meet their intended targets, adult vaccination remains often below the desired coverage level. In the general Belgian adult population, 62% was vaccinated against tetanus in 2008, and diphtheria-tetanus coverage ranged from 61 to 74% in four other European countries [4,5]. Adult pertussis vaccination coverage is not assessed in Belgium. In high risk patients, it is often challenging to reach a high uptake [6]. These patients are usually followed by a specialist and may therefore visit less often an occupational physician or general practitioner, who is usually in charge of vaccination. Circulating antibodies are needed for protection at the time of exposure to toxins, certainly in the case of tetanus and diphtheria [7,8]. However, protective titers are not always reached in at-risk patients because vaccine immune responses might be impaired [9,10]. Since tetanus is not transmitted from human to human, individual vaccination is the only mode of protection since the principle of herd immunity does not apply. In contrast, vulnerable individuals may benefit from herd immunity when a large proportion of the population is protected against diseases such as diphtheria and pertussis. Unfortunately, relatively few people from the general population have protective titers against these diseases due to waning immunity [11–15]. Moreover, cases of pertussis and diphtheria have resurged in the past few years, albeit more sporadically for diphtheria [14,16]. Despite these health risks, serosurveillance studies in the general population and patient groups have been sparse to date. In the present study we assessed seroprotection against diphtheria, tetanus and seroprevalence of pertussis antibodies in at-risk patients in a tertiary care hospital in Belgium.

Methods

Study procedure and population

The present study is a monocentric cross-sectional serosurvey in adult at-risk patients attending the University Hospitals of Leuven. This is a tertiary referral hospital in Belgium, which has about 1800 beds and covers approximately 700 000 outpatient consultations annually [17,18]. All patients older than 18 years who attended the outpatient clinic because of a previous diagnose of diabetes mellitus (DM), heart failure, chronic pulmonary obstructive disease (COPD), chronic kidney disease (CKD), HIV or solid organ transplant (SOT) of lung or heart during a consecutive 5-month recruitment window between September 2014 and March 2016 were invited to the study. Signed informed consent was obtained from all participants. Data were collected with a structured patient interview on vaccination status, disease characteristics and severity, and demographic and socio-economic background. Detailed data on the larger survey on vaccination status and determinants of vaccination are reported elsewhere [19]. Disease severity was classified according to international guidelines (the Global Initiative for Chronic Obstructive Lung Disease (GOLD) for COPD, Kidney Disease Improving Global Outcomes (KDIGO) for CKD, and the New York Heart Classification (NYHA) for heart failure) [20–22]. Severe disease state was defined as KDIGO≥4, GOLD stage C or D and NYHC class 3 or 4. Documented proof of vaccination was required for calculation of coverage rates. Vaccination data were retrieved from documents provided by patients, medical records of the general practitioner, or the Flemish vaccination register. Patients were considered correctly vaccinated against diphtheria and tetanus if they received the vaccine within in the last 10 years. Since adult pertussis vaccination was only recommend in Belgium since 2013, no time restriction was imposed for the definition of correct pertussis vaccination and having received at least once a pertussis containing vaccine at adult age or within the past 10 years was thus sufficient. The study was performed in accordance with the ethical standards of the Helsinki Declaration and approved by the Ethics Committee Research UZ/KU Leuven of Leuven, Belgium (S56765).

Laboratory methods

Blood samples were centrifuged for 10 minutes at 1942 g after coagulation at room temperature. Following centrifugation, serum aliquots were stored at -20°C until serological analysis with a magnetic bead-based Luminex multiplex assay for determination of IgG antibodies against diphtheria toxin (DT), tetanus toxin (TT), pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (Prn) at the Belgian scientific institute of public health (Sciensano) [23]. The lower limit of quantification (LLOQ) was defined as the lowest concentration within the linear part of the standard curve and corresponds to a sample to blank ratio ≥3, adjusted upwards to obtain a meaningful threshold. LLOQ was 0.01 IU/ml for anti-TT, 0.001 IU/ml for anti-DT, 0.5 IU/ml for anti-PT and 1 IU/ml for anti-FHA and anti-Prn. Anti-DT and anti-TT titers <0.01 IU/ml were considered seronegative and those ≥0,1 IU/ml seroprotective [24]. For pertussis no correlate of protection has been defined, but the presence of circulating antibodies is related to protection [25]. Anti-PT, anti-FHA and anti-Prn titers ≥5 IU/ml were used as cut-off value for pertussis seropositivity. Since especially anti-PT is considered to be important for protection, seropositive anti-PT titers were used as indication for pertussis immunity [26]. Anti-PT titers ≥50 IU/ml as indicative for pertussis infection or vaccination in the past 2 years and anti-PT titers ≥100 IU/ml as indicative for a

recent infection or vaccination. A limitation to this assessment, is the unknown origin of pertussis antibodies, i.e. from vaccination or from natural infection.

Statistical analysis

Geometric means titers (GMTs) and 95% confidence intervals (95%CI) were calculated from the logarithm of antibody titers and transformed back to the measurement scale. Antibody titers below the LLOQ were replaced by LLOQ divided by two for the calculation of GMTs and confidence intervals. The prevalence rates of seroprotection against tetanus and diphtheria and seroprevalence of pertussis antibodies are reported with exact binomial 95% confidence intervals (95%CI). Determinants (disease type, vaccination status and demographic characteristics) of seroprotection against diphtheria and tetanus and anti-PT seropositivity were analyzed with multiple logistic regression. Subsequently, socio-economic characteristic were also added to the model. Time since vaccination controlled for age and sex was analyzed separately within the group of vaccinated patients. A test probability of 5% was considered statistically significant. All data were analyzed with R. version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, 2013).

Results

Patient characteristics

A total of 1331 patients participated in the vaccination coverage study, of whom 1052 (85.5%) gave additional consent for blood sample collection. The present analysis is limited to these patients (66.9% males), whose characteristics are shown in table 1. A severe disease state was present in all CKD patients, 57.6% of COPD patients, and in 41.6% of heart failure patients. In the HIV group, 98.0% had a CD4+ count of ≥200 cells/mm² and 45.4% were men who have sex with men (MSM). Of the 230 SOT patients, 128 patients had received a lung transplantation and 127 a heart transplantation. Overall, less than one third of the patients was correctly vaccinated against diphtheria-tetanus.

Table 1: characteristics of study participants

	All patients (n=1052)	DM type 1 (n=172)	DM type 2 (n=77)	CKD (N=130)	COPD[a] (N=170)	Heart failure[b] (N=77)	HIV (n=196)	SOT (n=230)
Personal data	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Female gender	348 (33.1)	80 (46.5)	25 (32.5)	43 (33.1)	54 (31.8)	20 (26.0)	53 (27.0)	73 (31.7)
Median age, years (range)	59 (18-92)	44 (18-83)	67 (31-89)	73 (21-91)	65 (29-89)	70 (32-89)	46 (18-75)	59 (19-87)
Age								
< 40 years	173 (16.4)	66 (38.4)	2 (2.6)	5 (3.8)	1 (0.6)	2 (2.6)	62 (31.6)	35 (15.2)
40-64 years	492 (46.8)	81 (47.1)	30 (39.0)	25 (19.2)	81 (47.6)	25 (32.5)	125 (63.8)	125 (54.3)
≥ 65 years	387 (36.8)	25 (14.5)	45 (58.4)	100 (76.9)	88 (51.8)	50 (64.9)	9 (4.6)	70 (30.4)
Smoking								
Smoker	166 (15.8)	30 (17.4)	15 (19.5)	16 (12.3)	30 (17.6)	7 (9.1)	55 (28.1)	13 (5.7)
Ex-smoker	471 (44.8)	47 (27.3)	31 (40.3)	58 (44.6)	132 (77.6)	42 (54.5)	45 (23.0)	116 (50.4)
Net family income								
<1500 euro	225 (21.4)	23 (13.4)	8 (10.4)	30 (23.1)	48 (28.2)	21 (27.3)	52 (26.5)	43 (18.7)
1500-3000 euro	506 (48.1)	85 (49.4)	60 (77.9)	55 (42.3)	83 (48.8)	41 (53.2)	70 (35.7)	112 (48.7)
>3000 euro	217 (20.6)	63 (36.6)	7 (9.1)	15 (11.5)	14 (8.2)	9 (11.7)	64 (32.7)	45 (19.6)
Unkown income	104 (9.9)	1 (0.6)	2 (2.6)	30 (23.1)	25 (14.7)	6 (7.8)	10 (5.1)	30 (13.0)
Educational degree[c] (years of study)								
Lower education (<12 years)	355 (33.7)	20 (11.6)	38 (49.4)	64 (49.2)	74 (43.5)	43 (55.8)	45 (23.0)	71 (30.9)
Secondary education (12 years)	369 (35.1)	73 (42.4)	25 (32.5)	40 (30.8)	58 (34.1)	19 (24.7)	74 (37.8)	80 (34.8)
Higher education (>12 years)	320 (30.4)	79 (45.9)	14 (18.2)	24 (18.5)	38 (22.4)	15 (19.5)	75 (38.3)	75 (32.6)
Unknown education	8 (0.8)	0 (0)	0 (0)	2 (1.5)	0 (0)	0 (0)	2 (1.0)	4 (1.7)
Origin[d]								
Belgian	879 (83.6)	150 (87.2)	68 (88.3)	125 (96.2)	155 (91.2)	71 (92.2)	113 (57.7)	197 (85.7)
European	93 (8.8)	14 (8.1)	5 (6.5)	5 (3.8)	14 (8.2)	3 (3.9)	19 (9.7)	33 (14.3)
Non-European	80 (7.6)	8 (4.7)	4 (5.2)	0 (0)	1 (0.6)	3 (3.9)	64 (32.7)	0 (0)
Disease data								
Relevant comorbid disease[e]	205 (19.5)	9 (5.2)	6 (7.8)	43 (33.1)	34 (20.0)	24 (31.2)	19 (9.7)	70 (30.4)
Years since diagnosis/ transplantation (median (range))	8 (0-64)	18 (0-59)	13 (0-64)	4 (1-47)	7 (0-39)	6 (0-51)	8 (0-30)	7 (1-29)
Vaccination status								
Diphtheria-tetanus in the past 10 years	29.1 (26.4-32.0)	26.2 (19.9-33.5)	29.9 (20.2-41.5)	23.1 (16.3-31.4)	34.1 (27.1-41.8)	37.7 (27.1-49.5)	30.6 (24.3-37.7)	26.5 (21.0-32.8)
Any reported pertussis vaccine	9.3 (7.7-11.3)	12.8 (8.4-18.9)	10.4 (4.9-20.0)	4.6 (1.9-10.2)	10.6 (6.6-16.5)	14.3 (7.7-24.5)	3.6 (1.6-7.5)	11.3 (7.7-16.3)

[a] Patients were classified in categories of disease severity according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages: 20.0 % had GOLD stage A, 22.4% GOLD stage B, 9.4% GOLD stage C and 48.2% GOLD stage D. The severity of symptoms is measured with the Modified Medical Research Council Dyspnea Scale (mMRC) and the COPD Assessment Test (CAT). Patients with GOLD A and B are at low risk (0-1 exacerbation per year, not requiring hospitalization), GOLD C and D are high risk patients (≥2 exacerbations per year, or one or more requiring hospitalization). GOLD A and C have few symptoms (mMRC 0-1 or CAT <10), GOLD B and D have more symptoms (mMRC≥ 2 or CAT≥ 10) [20].
[b] Patients were classified in categories of disease severity according to New York Heart Classification (NYHA): 26.0% had class I (no limitation in ordinary physical activity), 32.5% class II (Mild symptoms and slight limitation during ordinary activity and comfortable at rest), 40.3% had class III (Marked limitation in activity due to symptoms, even during less-than-ordinary activity and comfortable only at rest) and 1.3% had class IV (severe limitations and experiences symptoms even while at rest) [21])
[c] Education: Lower Education = no secondary school diploma, Secondary education = secondary school diploma achieved, Higher education= university or university college diploma achieved
[d] European = At least one of the parents from European geographical area but not from Belgium, Non-European = At least one of the parents was not from the European geographical area.
[e] Relevant comorbidity is defined as having a comorbid disease that might influence vaccine-induced immunity (metabolic disease, systemic disease immunodeficiencies, renal disease).
CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, DM: Diabetes mellitus, SOT: solid organ transplantation

Seroprotection and seroprevalence

In total, 2.4% of antibody titers against TT, 35.6% against DT, 9.2% against PT, 1.7% against FHA and 6.0% against Prn were below the LLOQ. The GMTs, proportion of seroprotected and seronegative subjects for diphtheria and tetanus and prevalence of pertussis antibodies are shown in table 2. Seroprotective titers were reached in 83% of patients for tetanus and in 29% for diphtheria. Furthermore, 36% were seronegative (<0.01 IU/ml) for diphtheria and 2% for tetanus. About half of the patients (46%) had anti-PT antibodies, 8% had anti-PT titers indicative for infection or vaccination in the past few years and 2% had titers indicative for recent infection or vaccination (table 2). Overall, 13.9% of patients were seroprotected against tetanus and diphtheria and were anti-PT seropositive. Among the different patient groups, CKD patients had the lowest proportion of subjects with protection against tetanus and patients with COPD against diphtheria. Patients with heart failure had the lowest rate of seropositivity for pertussis.

Among the vaccinated patients, 36.6% were protected against diphtheria, 89.9% against tetanus, 67.3% were seropositive for anti-PT, 16.3% had titers indicative of pertussis infection or vaccination in the past few years and 5.1% titers indicative of infection or vaccination in the past few months. Among the not correctly vaccinated patients, 25.7% were protected against diphtheria, 79.8% against tetanus, 43.7% had an anti-PT titer ≥5IU/ml, 6.6% had titers indicative of infection in the past few years and 1.7% titers indicative of infection in the past few months.

Table 2: Geometric mean titers (GMTs) and seroprevalence of antibodies against tetanus toxin, diphtheria toxin, pertussis toxin, pertactin and filamentous hemagglutinin

	Reference	All patients (n=1052)	DM type 1 (n=172)	DM type 2 (n=77)	CKD (N=130)	COPD (N=170)	Heart failure (N=77)	HIV (n=196)	SOT (n=230)
GMT (IU/ml) ,95%CI)									
Anti-DT	-	0.01 (0.01-0.02)	0.04 (0.03-0.06)	0.01 (0.00-0.01)	0.01 (0.01-0.01)	0.01 (0.00-0.01)	0.01 (0.01-0.02)	0.04 (0.03-0.05)	0.01 (0.01-0.01)
Anti-TT	-	0.54 (0.49-0.61)	1.38 (1.13-1.67)	0.40 (0.27-0.58)	0.31 (0.22-0.43)	0.48 (0.37-0.62)	0.57 (0.40-0.83)	0.52 (0.41-0.65)	0.47 (0.37-0.60)
Anti-PT	-	4.21 (3.83-4.64)	3.97 (3.09-5.10)	5.33 (3.80-7.48)	5.13 (3.89-6.78)	4.52 (3.53-5.79)	3.72 (2.71-5.09)	3.57 (2.87-4.44)	4.14 (3.38-5.07)
Anti-FHA	-	22.7 (21.0-24.5)	22.6 (19.0-26.9)	29.4 (22.9-37.8)	32.1 (26.3-39.5)	26.3 (22.2-31.2)	37.3 (29.3-47.6)	15.6 (12.9-18.9)	17.9 (14.9-21.5)
Anti-Prn	-	9.94 (9.00-11.0)	19.1 (14.7-24.9)	7.90 (5.70-11.0)	6.81 (5.18-8.95)	9.86 (7.96-12.6)	14.5 (9.67-21.8)	8.58 (6.95-10.6)	8.20 (6.70-10.0)
Seroprotection		% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)
Diphtheria	≥ 0.1 IU/ml	28.9 (26.2-31.7)	45.9 (38.3-53.7)	22.1 (13.4-33.0)	21.5 (14.8-29.6)	17.1 (11.7-23.6)	22.1 (13.4-33.0)	41.3 (34.4-48.6)	23.0 (17.8-29.0)
Tetanus	≥ 0.1 IU/ml	82.6 (80.2-84.8)	95.3 (91.0-98.0)	79.2 (68.5-87.6)	72.3 (63.8-79.8)	80.0 (73.2-85.7)	83.1 (72.9-90.7)	85.2 (79.4-89.9)	79.6 (73.8-84.6)
Seronegativity		% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)
Diphtheria	< 0.01 IU/ml	35.6 (32.7-38.6)	20.9 (15.1-27.8)	50.6 (39.0-62.2)	43.8 (35.2-52.8)	48.2 (40.5-56.0)	36.4 (25.7-48.1)	19.4 (14.1-25.6)	41.3 (34.9-48.0)
Tetanus	< 0.01 IU/ml	2.4 (1.5-3.5)	0.6 (0.01-3.2)	1.3 (0.03-7.0)	6.2 (2.7-11.8)	2.4 (0.6-5.9)	0.00 (0.00-4.7)	2.0 (0.6-5.1)	3.0 (1.2-6.2)
Pertussis seroprevalence		% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)
Anti-PT	≥ 5 IU/ml	45.9 (42.9-49.0)	44.8 (37.2-52.5)	55.8 (44.1-67.2)	53.1 (44.1-61.9)	47.1 (39.4-54.9)	31.2 (21.1-42.7)	42.9 (35.8-50.1)	46.1 (39.5-52.8)
Anti-FHA	≥ 5 IU/ml	89.3 (87.2-91.1)	90.1 (84.6-94.1)	93.5 (85.5-97.9)	93.8 (88.2-97.3)	95.3 (90.9-97.9)	100.0 (95.3-100.0)	82.7 (76.6-87.7)	82.2 (76.6-86.9)
Anti-Prn	≥ 5 IU/ml	64.3 (61.3-67.2)	78.5 (71.6-84.4)	58.4 (46.6-69.6)	57.7 (48.7-66.3)	64.1 (56.4-71.3)	75.3 (64.2-84.4)	61.2 (54.0-68.1)	58.3 (51.6-64.7)
Pertussis infection or vaccination in last 2 years	≥ 50 IU/ml	7.5 (6.0-9.3)	7.6 (4.1-12.6)	7.8 (2.9-16.2)	9.2 (4.9-15.6)	9.4 (5.5-14.8)	7.8 (2.9-16.2)	5.6 (2.8-9.8)	6.5 (3.7-10.5)
Recent pertussis infection or vaccination	≥ 100 IU/ml	2.0 (1.2-3.0)	1.2 (0.1-4.1)	1.3 (0.03-7.0)	2.3 (0.5-6.6)	3.5 (1.3-7.5)	1.3 (0.03-7.0)	1.5 (0.3-4.4)	2.2 (0.7-5.0)

DT: diphtheria toxin, TT: tetanus toxin, PT: pertussis toxin, FHA: filamentous hemagglutinin, Prn: pertactin, CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, DM: diabetes mellitus, SOT: solid organ transplantation, CI: Confidence Interval

Determinants of seroprotection and seroprevalence

The association between seroprotection against DT and TT or anti-PT seropositivity and vaccination status, disease type, gender and age in adults with chronic diseases are shown in table 3. For diphtheria, seroprotection increased with recent vaccination and decreased with age. The seroprotection rate was also lower in all disease groups when compared to DM type 1, but this was only statistically significant for COPD and SOT (table 3). Protective titers for tetanus were more often attained when correctly vaccinated, and less often in woman, and in the oldest age group. The seroprotection rate was significantly lower in all disease groups when compared to DM type 1 (table 3).

As expected for pertussis, vaccinated patients were significantly more often seropositive or more likely to have a titer indicative of an infection or vaccination during the past 2 years. There was a similar trend for titers that indicate a recent infection or vaccination (p=0.06). Women were also more likely to have a titer indicative of recent infection or vaccination (table 3).

The addition of smoking, family income, education and origin in the analyses had a negligible effect on these results (data not shown in table 3), except for the effect of vaccination on titers indicative for recent pertussis exposure or vaccination (the OR increases to 3.6; 1.1-10.5; p = 0.03). These analyses further revealed that a European origin other than Belgian was associated with better protection against diphtheria (OR vs. Belgian: 2.1; 1.3-3.3; p<0.01). For tetanus, a net family income of >3000 euros was associated with better protection (OR vs. 1500-3000 euro: 1.9; 1.0-3.6; p<0,05), and a non-European origin with less protection (OR vs. Belgian: 0.4; 0.2-0.8; p<0.01). For pertussis, past smoking was associated with a seropositive titer (anti-PT ≥5IU/ml) (OR vs. non-smoking:1.4; 1.1-2.0; p = 0.03) and with a titer indicative for previous infection or vaccination (anti-PT ≥50IU/ml) (OR vs. non-smoking:1.8; 1.0-3.5; p=0.05) and active smoking was associated with titers indicative for a recent infection or vaccination (anti-PT ≥100IU/ml) (OR vs. non-smoking: 4.5;1.2-18.3; p=0.03).

Time since vaccination

Within the subgroup of patients who were vaccinated less than 10 years before the study we did not observe an effect of time since vaccination on protective titers for tetanus (OR:0.96; 0.84–1.1; p=0.55) or diphtheria (OR:0.9; 0.9-1.0; p=0.11). For pertussis, the number of years since vaccination was significantly associated with decreased odds for a seropositive anti-PT titer (≥5IU/ml) (OR:0.8; 0.7-0.9; p=0.002), past infection or vaccination (anti-PT ≥50IU/ml) (OR:0.7; 0.4–0.9; p=0.03).

Table 3: Determinants (disease type, vaccination status and demographic characteristics) of seroprevalence for diphtheria, tetanus and of pertussis: Multiple logistic regression

n=1052	Diphtheria (≥0.1IU/ml) OR (95%CI)	Tetanus (≥0.1IU/ml) OR (95%CI)	Pertussis (≥5IU/ml) OR (95%CI)	Pertussis (≥50IU/ml) OR (95%CI)	Pertussis (≥100IU/ml) OR (95%CI)
Age					
< 40 years	Reference	Reference	Reference	Reference	Reference
40-64 years	**0.3 (0.2-0.5)*** **	0.7 (0.3-1.2)	0.9 (0.6-1.3)	1.0 (0.5-2.0)	0.8 (0.2-3.1)
≥ 65 years	**0.2 (0.1-0.3)*** **	**0.2 (0.1-0.4)*** **	0.8 (0.5-1.3)	0.6 (0.3-1.4)	0.2 (0.0-1.2)°
(Correctly) vaccinated [§]	**1.8 (1.3-2.4)*** **	**2.4 (1.6-3.7)*** **	**2.8 (1.8-4.5)*** **	**2.7 (1.4-4.8)** **	2.9 (0.9-8.0)°
Disease group					
DM type 1	Reference	Reference	Reference	Reference	Reference
DM type 2	0.6 (0.3-1.2)	**0.3 (0.1-0.7)** **	1.7 (1.0-3.1)°	1.4 (0.5-3.9)	2.7 (0.1-32.2)
CKD	0.7 (0.4-1.3)	**0.2 (0.1-0.5)*** **	1.6 (1.0-2.7)°	2.0 (0.8-5.0)	7.2 (1.0-65.7)°
COPD	**0.4 (0.3-0.7)** **	**0.3 (0.1-0.6)** **	1.2 (0.8-1.9)	1.6 (0.7-3.8)	6.2 (1.2-48.0)*
Heart failure	0.6 (0.3-1.2)	**0.4 (0.1-1.0)***	0.6 (0.3-1.0)°	1.3 (0.4-3.8)	2.9 (0.1-34.5)
HIV	0.8 (0.5-1.3)	**0.2 (0.1-0.4)*** **	1.0 (0.7-1.5)	0.8 (0.4-2.0)	2.0 (0.3-15.9)
SOT	**0.5 (0.3-0.7)** **	**0.2 (0.1-0.4)*** **	1.1 (0.7-1.7)	1.0 (0.4-2.2)	3.0 (0.6-21.5)
Female (vs. male)	1.0 (0.7-1.3)	**0.3 (0.2-0.5)*** **	0.9 (0.7-1.2)	1.2 (0.7-2.0)	**3.4 (1.4-8.8)** **

CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, DM: diabetes mellitus, SOT: solid organ transplantation.
[§] Correctly vaccinated against diphtheria and tetanus: ≥1 dose within in the last 10 years. Correctly vaccinated against pertussis: ≥1 dose with a pertussis containing vaccine at adult age or within the past 10 years. °p< 0.1, *p<0.05, **p<0.01, ***p<0.001.

Discussion

Our study on seroprevalence of antibodies demonstrates that a large group of at-risk patients remain susceptible to vaccine-preventable diseases. Compared to currently available data, the study provides a comprehensive insight into the seroprotective status of clinical at-risk groups because of the diversity of clinical conditions and number of patients that were included.

We found a seroprotective status for tetanus in 83% of patients, but only 29% reached protective titers for diphtheria and 46% were anti-PT seropositive. Overall, less than 15% of the patients were protected against tetanus and diphtheria and anti-PT seropositive. These numbers are considerably lower than corresponding data from the general Belgian population [11,12]. In a seroprevalence study from 2006, Theeten et al. reported seroprotective levels for tetanus in more than 90% of persons aged >40 years and for diphtheria in 55% of persons aged 1-65 years [11]. Van der Wielen et al. found a seroprevalence of anti-PT antibodies (≥5IU/ml) against pertussis in about 70% of those between 1 and 65 years of age in 1993-94 [12]. Although the comparison might be hampered due to changes in the vaccination programs over the years, the use of different age groups and the lack of vaccination data in population studies, some reasons for these differences can be suggested. A major factor could be the low vaccination uptake in patients. We found that less than 30% was correctly vaccinated against diphtheria and tetanus, which is less than half the coverage rate in the general Belgian population [27]. We only used documented vaccination data, and as

such may have underestimated the coverage rates in our study population. Nevertheless, we found that correct vaccination predicts higher seroprotection or seroprevalence rates for all studied diseases.

Time since last vaccination can also influence the level of immunity against vaccine-preventable disease. Evidence exist that antibody titers wane even faster in high-risk groups. Studies in transplant and CKD patients show an accelerated decline, particularly in diphtheria antibodies compared to tetanus antibodies [28,29]. Also HIV patients, even those with RNA-HIV below 50 copies/ml sustain less antibodies due to an impaired cellular immune response [30]. However, we could only find an effect of time since vaccination on the protection against pertussis, for which immunity after both vaccination and natural infection is known to be rather short-lived [13]. Therefore, a booster every 10 years might be sufficient to maintain immunity for tetanus and diphtheria, but not for pertussis in this population.

It remains striking, however, that only 38% of the vaccinated patients had protective titers against diphtheria. The exact impact of chronic disease on vaccine immunology is complex, incompletely studied and influenced by many factors, such as the characteristically older age of at-risk patients, comorbidities, disease severity and treatment.

Ageing goes along with suppression of innate and adaptive immune reactions, or immunosenescence, and leads to a reduced immune response to vaccination [31]. Consistent with other studies, we found age to be a negative predictor for the seroprevalence of antibodies against diphtheria and tetanus [14,15]. However, as for pertussis seroprevalence, age could not be defined as a predictive factor. In addition to the influence of vaccination, it is also likely that antibodies were evoked or boosted by natural infection since pertussis has been increasing in Belgium since 2011 [32,33]. Although the seroprevalence of pertussis antibodies is significantly associated with vaccination, the pertussis vaccination coverage is less than 10%. Therefore, we assume that many patients with the high antibody titers had been exposed to wild-type pertussis.

Increased susceptibility might also be related to the immunosuppressive characteristics of chronic disease or the use of immunosuppressive treatment. Rafi et al. showed that increased chronic disease burden may go along with decreased cell-mediated immunity, which in turn might affect humoral immunity [34]. Among the different disease groups in our study, patients with CKD, HIV and SOT had the lowest odds of being protected against tetanus and patients with COPD and SOT the lowest odds for protection against diphtheria. Among SOT patients, the induction and maintenance of antibodies upon vaccination might be reduced due to the immunosuppressive treatment they take to avoid graft rejection [35]. In COPD and CKD patients, vaccine immunology can be impaired by disrupted innate and adaptive immune responses caused by chronic inflammation of the airways and uremic state, respectively [36–38]. For HIV patients, reduced vaccine immune response is mostly seen in those with a low CD4-cell count, a detectable viral load and in those not using anti-retroviral therapy. In our study on the other hand, nearly all patients had CD4+ counts ≥200 cells/µl.

Finally, we also observed some gender differences. Firstly, men were significantly better protected against tetanus than women. The difference has been linked to vaccination practice during the military service and to vaccination after manual work related injuries [14].

We also looked at titers suggestive of recent pertussis infection or vaccination. In total, 8% had a PT-IgG titer suggestive of an infection or vaccination in the past few years and 2% of a more recent infection or vaccination. Patients with COPD were more likely to have had a recent infection or vaccination compared to DM type 1 patients. This is consistent with another study where the seroprevalence of anti-PT was higher in COPD patients compared to healthy controls [39]. Also (past) smoking was associated with pertussis infection or vaccination. Given the low vaccination uptake, this enforces the assumption that both COPD patients and smokers are predisposed to develop respiratory infections such as pertussis due to a reduction of protective functions in the airway epithelium [3,40]. Recent pertussis infections or vaccination were also mainly seen in women, which is in accordance with the observation of predominant occurrence of pertussis in women in the general population [41].

Given the high susceptibility of at-risk patients, we advocate for a close follow-up of their vaccination status. Vaccination is the best available tool to prevent infectious diseases, even when vaccine immunity is reduced due to age, disease or treatment. It still has the added value of reducing the likelihood of acquiring severe disease, such as pertussis infection requiring hospitalization or resulting in post-tussive vomiting [1,42]. Since patients with chronic disease are often followed by a specialist, an appropriate recommendation by the treating specialist may convince them to get their vaccine with their general practitioner. In addition, it remains equally important to avoid transmission of infectious pathogens to vulnerable patients by vaccinating their close contacts and by implementing universal vaccination programs.

Some limitations of our study should be mentioned. The study was performed in a single, albeit large, tertiary care hospital, which may limit extrapolation of the results to all at-risk patients. A general limitation inherent to all seroprevalence studies is the unknown origin of anti-pertussis antibodies, i.e. from vaccination or from natural infection. However, the vaccination coverage was below 10%. We believe that lack of documentation plays a limited role since adult booster vaccination were only recommended since 2013 and cocoon vaccination since 2009, but compliance with these strategies was rather low before and during the recruitment of patients [43]. Unfortunately, we could not include a lifetime history of vaccination (or exposure) as these data could not be reliably collected. Finally, the cut-off values for recent pertussis infection were applied in accordance with international agreements, but patients with chronic diseases may mount less antibodies due to their disease or immunosuppressive therapy.

In conclusion, our data show that patients with chronic diseases are at increased risk for vaccine preventable diseases. Noticeably 17% of patients remain susceptible to tetanus, 71% to diphtheria and 54% have low titers to pertussis. This might be explained by the low vaccination coverage, age or the influence of disease and therapy on vaccine immunity. We recommend close follow-up of the vaccination

status in patients with chronic diseases and advocate additionally indirect protection through universal vaccination programs and vaccination of their direct contacts.

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CHAPTER 6: Seroprevalence of antibodies against vaccine-preventable diseases in children with chronic diseases

Manuscript in preparation

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Abstract

Background

Children with chronic diseases have an increased risk for complications following infections. We assessed the seroprevalence of antibodies against measles, mumps, rubella (MMR) and diphtheria, tetanus, pertussis (DTP) to evaluate the adequacy of current vaccination programs to protect children with chronic diseases against these vaccine-preventable diseases.

Methods

Antibody titers were determined with ELISA (MMR) and multiplex assay (DTP) in 222 children, aged 2-21 years, with allergies (n=14), congenital heart disease (n=25), diabetes type 1 (n=58), cystic fibrosis (n=9), primary immunodeficiency (n=88) or a history of solid organ transplantation (SOT) (n=28). Vaccination data were retrieved from documents provided by the parents, general practitioners and verified against the Flemish vaccination registry (Vaccinnet). Factors associated with seroprevalence were analyzed with logistic regression.

Results

The seroprevalence of antibodies was 83.3% for measles (≥150mIU/ml), 82.9% for mumps (≥230 labU/ml) and 80.6% of children were protected against rubella (≥10IU/ml). Most patients were protected against tetanus (≥0.1IU/ml; 93.2%), but only 61.3% were protected against diphtheria (≥0.1IU/ml) and 53.2% had antibodies (≥5 IU/ml) against pertussis. SOT patients had the lowest seroprevalence rates for all studied vaccine-preventable diseases except tetanus. Age-appropriate vaccination was associated with a higher seroprevalence, albeit not significant for diphtheria and pertussis.

Conclusion

Overall, 7% (tetanus) to 47% (pertussis) of children with chronic disease remained susceptible to vaccine-preventable diseases. This is partly explained by a relatively low vaccination coverage (73% for DTP to 85% for MMR) and possibly also by the impact of chronic diseases and associated treatment on the immune system. Our findings highlight the importance of close follow-up of the vaccination status in children with chronic diseases.

Keywords: seroprevalence, seroprotection, children with chronic disease, vaccination, immunity, diphtheria, tetanus, pertussis, measles, mumps, rubella

Introduction

The past decade has seen several outbreaks with vaccine-preventable diseases such as measles, mumps and pertussis. These outbreaks are threats to children with chronic disease who are at increased risk of complications due to their underlying condition or immunosuppressive treatment. For example, an Italian study showed that children with underling conditions had a 10-fold increased risk of being hospitalized with measles compared to healthy children [1]. Furthermore, a case-control study showed that patients with asthma had an almost twofold increased risk of pertussis infection [2]. Vaccination is the best available measure to avoid such complications.

In terms of diphtheria, tetanus and pertussis (DTP) vaccination, the Belgian National Immunization Technical Advisory Group (NITAG) recommends four doses during infancy, one booster during childhood (between 5 and 7 years) and one booster at the age of 13-14 years [3]. Whole cell pertussis-containing vaccines were used for infant doses until 1999, after which they were replaced by acellular pertussis-containing vaccines. Concerning measles, mumps and rubella (MMR) vaccination, a first dose is recommended at the age of 12 months and another one at the age of 10-11 years. Live-attenuated vaccines such as the MMR vaccine are, however, contra-indicated in severely immunocompromised children such as transplant recipients and some children with primary immunodeficiencies. Apart from these vaccines, the recommended childhood vaccination program in Belgium includes vaccination against polio, hepatitis B, *Haemophilus influenza* type b (all part of the hexavalent dTap-IPV-HBV-Hib vaccine), pneumococcal disease (13-valent conjugated pneumococcal vaccine), rotavirus and meningococcal disease from serogroup C.

To date it remains unknown whether these recommendations sufficiently protect children with chronic diseases. There are some issues that might disrupt protection in these patients. Firstly, children with a chronic disease could be more likely to miss out on prescheduled vaccination visits. During a survey on vaccination coverage in Flanders (Belgium), illness was a frequently reported reason for missing vaccination among unvaccinated children of the general population [4]. However, the vaccination coverage in children with chronic disease remains unknown. Secondly, the immunosuppressive nature of underlying condition or treatment might impair the immune responses to vaccination [5–7]. Thirdly, outbreaks of measles, mumps and pertussis in recent years indicate insufficient immunity at the population level, and hence insufficient herd immunity, due to below optimal vaccination coverage, waning immunity and/or development of new pathogenic strains [8]. Serosurveillance studies are essential to assess population immunity, but few have been published. A Belgian seroprevalence study in 2006 reported seroprevelance of 96.1% for measles and 89.6% for mumps and seroprotective levels in 87.6% for rubella and 55.2% for diphtheria in the 1 to 65 years old population [9]. For pertussis, Van der Wielen et al. reported in 1993-94 antibodies against pertussis toxin (PT), Pertactin (Prn) and filamentous hemagglutinin (FHA) in about 70%, 40% and 99% of persons, respectively [10]. These numbers are similar to seroprevalence data from other countries, such as the United States, Australia and Germany [11–14]. However, since those assessments in Belgium a pertussis adolescent booster has been introduced, childhood vaccination coverage has

markedly increased and disease epidemiology has changed. These changes make it currently unclear if at risk patients are protected by group immunity. In addition, data on the seroprevalence of vaccine-preventable disease in children are scarce. We therefore assessed the seroprevalence of antibodies against measles, mumps, rubella, diphtheria tetanus and pertussis, and the association with vaccination history, in children with chronic conditions in a tertiary care hospital in Belgium.

Methods

Study design and population

This is a cross-sectional serosurvey of children with chronic diseases in the university hospitals of Leuven. This is the largest tertiary care hospital of Belgium with about 1800 beds and 700 000 outpatient consultations per year [15,16]. Parents of children older than 18 months of age who attended the outpatient clinic between September 2014 and April 2016 for a follow-up of allergy, congenital heart disease, diabetes mellitus type 1, cystic fibrosis, primary immunodeficiency (PID) or solid organ transplantation (SOT) were approached to participate. To limit the burden of blood sample collection fragile children in some outpatient clinics were only asked for participation in the serosurvey if venipuncture was already scheduled as part of the routine follow-up. Signed informed consent form was obtained from all caregivers and informed assent form was requested from all children as of the age of seven years. Results from the larger survey on vaccination coverage and determinants will be reported elsewhere (Boey et al. unpublished data). Data were collected with a structured interview of the child's caregiver that included items on the socio-demographic background, vaccination history and disease characteristics. Vaccination data were transcribed from available documents at the time of the interview. If no documents were available at the time of the interview caregivers were requested to send a copy by mail. Additional vaccination data were retrieved from the records of the general practitioner and from the Flemish vaccination register (Vaccinnet). The study was approved by the Ethics Committee Research UZ/KU Leuven of Leuven, Belgium (S56765).

Definitions

Age-appropriate MMR vaccination was defined as having received at least one dose for children ≤11 years of age and 2 doses for children from their 12th birthday onwards. MMR doses were considered valid when administered after 50 weeks of age and at least 4 weeks apart. Age-appropriate vaccination against DT was defined as having received at least 4 doses in children up to their 8th birthday, at least 5 doses from the age of 8 years onwards, and at least 6 doses from the 16th birthday onwards. The same definition applies to pertussis, except that a fifth dose was only required in children born after 1998 because this vaccine has only been recommended since 2004.

Laboratory assessment

Blood samples were centrifuged for 10 minutes at 3000 rmp after coagulation at room temperature. Following centrifugation, serum aliquots were stored at -20°C until serological analysis at the Belgian national scientific institute of public health (Sciensano).

Measles, mumps and rubella antibodies were determined with enzyme-linked immunosorbent assays (ELISA) (Enzygnost, Siemens) according to the manufacturer's instructions using an automated pipetting BEP-2000 (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) system. These tests have a sensitivity of 99.6% for measles, 95.4% for mumps and 100% for rubella and a specificity of 100% for measles, 93.7% for mumps and 98.5% for rubella antibodies. All samples were analyzed using the same kit lot. Samples were seronegative if the optical density (OD) was below 0.1, equivocal (gray zone) if the OD was between 0.1 and 0.2, and positive if the OD was above 0.2. After conversion to IU/ml according to the kit insert ($\log(\text{mIU/mL}) = \alpha \times \text{corrected } \Delta OD\beta$ with α and β as lot-specific constants), titers above 330 mIU/ml (measles), 487 labU/ml (mumps) and 6 IU/ml (rubella) were considered seropositive. Titers below 150 mIU/ml (measles), 230 labU/ml (mumps) and 5 IU/ml (rubella) were below the detection limit and considered seronegative. Titers between those cut-off values were considered equivocal. Rubella titers >10 IU/ml are correlated with protection whereas no agreement on correlate of protection has been made for measles and mumps [17,18].

Immunoglobulin G (IgG) antibodies against diphtheria toxin (anti-DT), tetanus toxin (anti-TT),anti-PT, anti-FHA and anti-Prn were determined with a magnetic bead-based Luminex multiplex assay, as previously described [19]. Anti-DT and anti-TT titers ≥ 0,1IU/ml were considered seropositive and seroprotective and titers <0.01 IU/ml seronegative. For pertussis no correlate of protection is defined, but the presence of circulating antibodies is related to protection [20]. Anti-PT, anti-FHA and anti-Prn titers ≥5 IU/ml were used as cut-off values for pertussis seropositivity. Since especially anti-PT is considered to be important for protection, seropositive anti-PT titers were used as indication for pertussis immunity [21]. Anti-PT titers ≥50 IU/ml were considered indicative for pertussis infection or vaccination in the past 2 years and anti-PT titers ≥100 IU/ml as indication for a recent infection or vaccination.

Statistical analysis

Geometric Mean Titers (GMTs) and associated confidence intervals were calculated from the logarithm of antibody titers and transformed back to the measurement scale for reporting. Titers below the lower limit of quantification of the Luminex test and below detection limit of the Enzygnost test were replaced by the respective limit divided by two. Seroprotection, seropositive, equivocal and seronegative rates are reported with an exact Clopper-Pearson 95% confidence interval.

Determinants of the seroprotection against rubella, tetanus and diphtheria were analyzed with logistic regression. Since there is no formal agreement on a correlate of protection for measles, mumps and pertussis, determinants of seroprevalence of antibodies were analyzed, using the same test. To this end, we used seropositive titers (≥5 IU/ml) for pertussis and merged equivocal and seropositive titers for measles (≥150 mIU/ml) and mumps (≥230 labU/ml), which is in agreement with the European Sero-Epidemiology Network (ESEN) studies [22,23]. PID patients on IgG-treatment were excluded from this analysis because antibodies might have been derived from the plasma donor. Two logistic regression models were fitted for each outcome: A univariate logistic regression model with age-appropriate vaccination as the sole predictor

of seroprotection or seroprevalence in all children, and a multiple logistic regression model to assess the combined effect of sex, the number of received doses, time since last vaccination, disease group (SOT versus PID versus all other groups), and relevant comorbidity in patients who received at least one dose of the relevant vaccine. A test probability of 5% or less was considered statistically significant. All data were analyzed with R. version 3.6 (R Foundation for Statistical Computing, Vienna, Austria, 2019).

Results

Study population

A total of 566 children participated in a vaccination coverage study of whom 222 (39.2%) gave additional consent for blood sampling. This included patients with allergies (n=14), congenital heart disease (n=25), diabetes (n=58), cystic fibrosis (n=9), PID (n=88) and SOT recipients (n=28). The basic characteristics regarding age, gender, comorbid disease and vaccination status are listed in table 1. About half of participants were boys. Age was grouped by age at last birthday, and ranged from 1 year to 21 years with a median of 10 years.

Among the 14 children with allergies, we recorded allergies against pollen (n=9), house-dust mite (n=6), food (n=8, including 5 children with egg allergy), pets (n=5), latex (n=2), insects (n=1) and medicinal drugs (n=1). All children with diabetes had type 1 diabetes mellitus. The large majority of patients with PID had a humoral immunodeficiency (n=68; (77%)) and about a third (n=27; (31%)) received IgG therapy. In the SOT group, 13 children received a kidney transplantation, 12 a liver transplantation, 2 a combined liver-small bowel-pancreas transplantation and 1 a combined kidney-liver transplantation.

In total 80.6% of children were age-appropriately vaccinated against MMR. In particular, 86.0% received the first dose and 83.0% of those ≥12 years received the second dose. For eight patients, proof was only obtained for the second dose without evidence of an earlier dose. Overall, 73.0% of patients were age-appropriately vaccinated against DT. Particularly, 96.8% received at least one dose of DT vaccine, 85.1% completed the infant schedule, 82.1% of children ≥7 years received the childhood booster and 60.9% of those ≥15 years received the adolescent booster. All recorded vaccines also contained an acellular pertussis component, except for one childhood dose, which was administered before the Tdap vaccine had become available.

Table 1: Patient characteristics

	All patients (n=222)	Allergy (n=14)	Congenital heart disease (n=25)	Diabetes (n=58)	Cystic fibrosis (n=9)	PID (n=88)	SOT (n=28)
Personal data	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*
Boys	123 (55.4)	12 (85.7)	8 (32.0)	34 (58.6)	4 (44.4)	52 (59.1)	13 (46.4)
Age (median(range), years)	10 (1 – 21)	6 (1 – 14)	13 (3 – 17)	13 (4 – 17)	15 (2 – 17)	6 (1 – 18)	14 (2 – 21)
≤ 7 years	82 (36.9)	8 (57.1)	4 (16.0)	7 (12.1)	3 (33.3)	53 (60.2)	7 (25.0)
8-11 years	46 (20.7)	2 (14.3)	6 (24.0)	10 (17.2)	0 (0.0)	22 (25.0)	6 (21.4)
> 11 years	94 (42.3)	4 (28.6)	15 (60.0)	41 (70.7)	6 (66.7)	13 (14.8)	15 (53.6)
Disease data							
Relevant comorbidity [a]	23 (10.4)	1 (7.1)	1 (4.0)	7 (12.1)	1 (11.1)	13 (14.8)	0 (0.0)
Age at diagnosis/ transplantation (median (range)) [b]	3 (0-19)	1 (0-12)	0 (0-9)	7 (0-16)	0 (0-11)	2 (0-14)	4 (0-19)
Vaccination status	*% (95%CI)*	*% (95%CI)*	*% (95%CI)*	*% (95%CI)*	*% (95%CI)*	*% (95%CI)*	*% (95%CI)*
Measles-mumps-rubella							
Age-appropriate vaccination [c]	80.6 (74.7-85.5)	78.6 (48.8-94.3)	72.0 (50.4-87.1)	86.2 (74.1-93.4)	88.9 (50.7-99.4)	86.4 (77.0-92.5)	57.1 (37.4-75.0)
Diphtheria-tetanus-pertussis [d]							
Age-appropriate vaccination [e]	73.0 (66.5-78.6)	71.4 (42.0-90.4)	72.0 (50.4-87.1)	72.4 (58.9-83.0)	55.6 (22.7-84.7)	77.3 (66.9-85.2)	67.9 (47.6-83.4)

[a] Relevant comorbidity is defined as having a comorbid disease that might influence vaccine-induced immunity (metabolic disease, systemic disease immunodeficiencies, renal disease)
[b] Three missing values
[c] Age-appropriate MMR vaccination was defined as having received at least one dose for children ≤11 years of age and 2 doses for children >11 years of age. The first MMR dose had to be administered as of the age of 50 weeks and the minimal interval between the two doses is 4 weeks.
[d] All infant doses and adolescent boosters contained a pertussis component, except for one childhood booster dose that was administered before the childhood pertussis booster recommendation came out in 2004.
[e] Age-appropriate vaccination against diphtheria and tetanus was defined as having received at least 4 doses for children ≤7 years of age, at least 5 doses for children >7 years and at least 6 doses for children >15 years.
PID: Pediatric immunodeficiency, SOT: Solid organ transplantation

Seroprotection and seroprevalence

GMTs are represented in table 2 and the proportion of seroprotective, seropositive, equivocal and seronegative titers are shown in figure 1. Seroprevalence of antibodies (seropositive or equivocal titers) was found in 83.3% of patients for measles, 77.9% for mumps and seroprotection against rubella was reached in 80.6%. Seroprotection was achieved in 61.3% for diphtheria and 93.2% for tetanus, and 53.2% was seropositive for anti-PT. Furthermore, 2.3% had an indication of recent pertussis infection or vaccination and 6.3% had an indication of pertussis infection or vaccination in the past few years.

Overall, the highest proportion of seronegative titers was observed in SOT patients. Among the PID patients who received IgG therapy, seroprevalence was 96.3% for measles and mumps (seropositive or equivocal) and rubella (seroprotective), 100% for tetanus (seroprotective), 96.3% diphtheria (seroprotectieve) and 92.6% for pertussis (anti-PT seropositive). On the contrary, among PID patients who did not receive IgG treatment, the seroprevalence of antibodies was only 77% for measles and mumps (seropositive or equivocal), 82.0% for rubella (seroprotective), 57.4% for diphtheria (seroprotective), 90.2% for tetanus (seroprotective) and 41% for pertussis (anti-PT seropositive).

Among the age-appropriately vaccinated patients, seroprevalence was observed 87.7% for measles (seropositive or equivocal), 83.8% for mumps (seropositive or equivocal) and in 85.5% for rubella (seropositive or equivocal), 63.6% (seroprotective), 95.7% for tetanus (seroprotective) and 55.8% for pertussis (anti-PT ≥5IU/ml). In contrast, among not-correctly vaccinated patients, only 60.5% were protected against rubella, 55.0% against diphtheria and 86.7% against tetanus. In addition, only 65.1% had antibodies against measles, 53.5% against mumps and 45.8% against pertussis.

Table 2: Geometric mean titers (GMTs) of antibodies against measles, mumps, rubella, tetanus toxin, diphtheria toxin, pertussis toxin, pertactin and filamentous hemagglutinin

GMT, 95% CI	All patients (n=222)	Allergy (n=14)	Congenital heart disease (n=25)	Diabetes (N=58)	Cystic fibrosis (N=9)	PID (n=88)	SOT (n=28)
Anti-measles (IU/ml)	638 (536-760)	744 (310-1782)	621(351-1098)	591(449-777)	2072 (995-4313)	706 (535-930)	356(215-591)
Anti-mumps (labU/ml)	597 (514 - 694)	632 (366 - 1092)	799 (496 - 1287)	515 (397 - 667)	444 (252 - 783)	741 (583 - 942)	340 (216- 535)
Anti-rubella (IU/ml)	20.6 (18.1 - 23.4)	22.6 (13.9 - 36.8)	19.0 (12.3 - 29.2)	19.0 (15.6 - 23.2)	20.6 (12.3 - 34.4)	24.6 (20.1 - 30.0)	14.2 (9.0 - 22.4)
Anti-DT[a]	0.12 (0.10-0.16)	0.08 (0.03-0.21)	0.12 (0.06-0.23)	0.11 (0.07-0.18)	0.16 (0.05-0.52)	0.17 (0.12-0.25)	0.06 (0.04-0.11)
Anti-TT(IU/ml)[a]	0.69 (0.57-0.83)	0.54 (0.30-0.98)	0.73 (0.38-1.42)	0.79 (0.54-1.17)	0.79 (0.21-2.94)	0.67 (0.50-0.88)	0.56 (0.35-0.90)
Anti-PT (IU/ml)[a]	4.77 (3.89-5.83)	5.46 (3.00-9.93)	5.53 (3.10-9.84)	4.96 (3.18-7.74)	6.18 (1.61-23.76)	5.50 (4.09-7.39)	2.11 (1.28-3.48)
Anti-FHA (IU/ml)[a]	36.0 (30.4 - 42.6)	43.5 (28.4 - 66.7)	48.2 (27.3 - 85.3)	46.7 (34.1 - 64.0)	40.3 (20.7 - 78.3)	32.9 (25.4 - 42.6)	18.6 (10.9 - 31.8)
Anti-Prn (IU/ml)[a]	26.5 (20.7 - 33.8)	23.5 (13.6 - 40.7)	29.5 (12.0 -72.7)	32.0 (18.6 - 55.0)	33.2 (9.7 - 113.6)	27.6 (19.2 - 39.9)	14.0 (8.3 - 23.6)

DT: diphtheria toxin, TT: tetanus toxin, PT: pertussis toxin, FHA: filamentous hemagglutinin, Prn: pertactin, PID: primary immunodeficiencies, SOT: solid organ transplantation, GMT: geometric mean titer. [a] Data from one PID patient missing

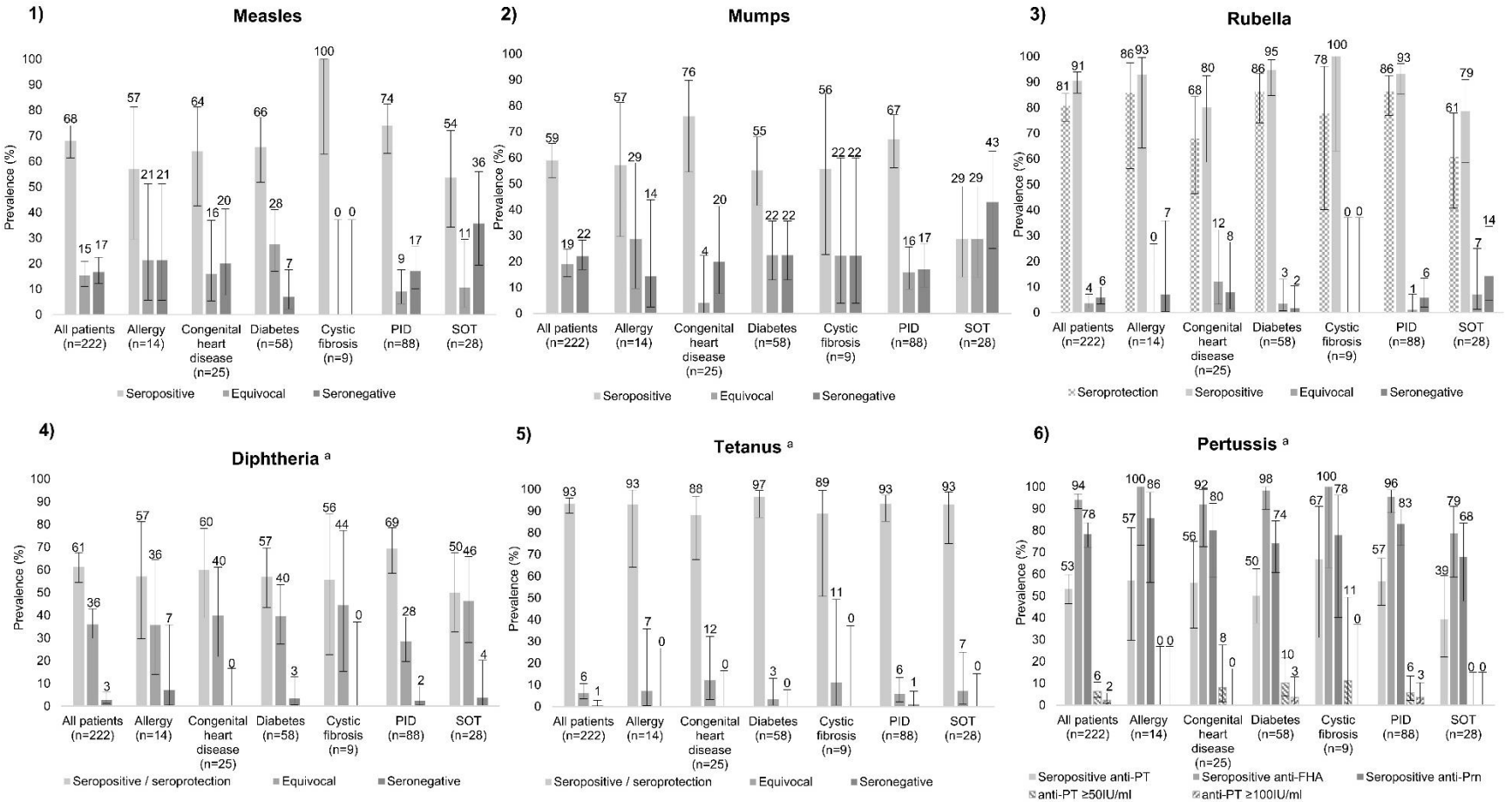

Figure 1: Seroprevalence (%, 95%CI) of antibodies against measles, mumps, rubella, tetanus toxin, diphtheria toxin, pertussis toxin, pertactin and filamentous hemagglutinin. Cut-off value 1) measles: seropositive ≥ 330 IU/ml, equivocal 150-330 mIU/ml, seronegative < 150 mIU/ml, 2) Mumps: Seropositive ≥ 487 labU/ml, Equivocal: 230-487 labU/ml, seronegative < 230 labU/ml, 3) Rubella: seroprotective ≥10 IU/ml, seropositive ≥ 6 IU/ml (also includes seroprotective), equivocal 5 IU/ml, seronegative < 5 IU/ml, 4) Diphtheria: Seropositive/seroprotective: ≥ 0.1 IU/ml, equivocal 0.01-0.1 IU/ml, seronegative < 0.01 IU/ml, 5) Tetanus: seropositive/seroprotective ≥ 0.1 IU/ml, equivocal 0.01-0.1 IU/ml, seronegative < 0.01 IU/ml, 6) Pertussis: seropositive anti-PT: ≥ 5 IU/ml, seropositive anti-FHA: ≥ 5 IU/ml, seropositive anti-Prn: ≥ 5 IU/ml, pertussis infection/vaccination in last 2 years: anti-PT ≥ 50 IU/ml, recent pertussis infection/vaccination: anti-PT ≥ 100 IU/ml. PT: pertussis toxin, FHA: filamentous hemagglutinin, Prn: pertactin, PID: primary immunodeficiencies, SOT: solid organ transplantation, GMT: geometric mean titer. [a] Data from one PID patient missing.

Determinants of seroprotection or seroprevalence

Simple logistic regression analysis showed that age-appropriate MMR vaccination increased the likelihood of having antibodies against measles (OR(95%CI): 4.2(1.9-9.4), p<0.001) and mumps (OR(95%CI): 5.1(2.4-11.0), p<0.001) and seroprotective titers for rubella (OR(95%CI): 4.3(2.0-9.3), p<0.001). Likewise, age-appropriate DT(P) vaccination was associated with seroprotection against tetanus (OR(95%CI): 3.2(1.1-9.7), p<0.05), but no significant effect was observed for diphtheria or pertussis.

Table 3 shows the results of a multiple logistic regression analysis of seroprotection or seroprevalence according to the number of received doses of the relevant vaccine, time since last vaccination and disease group (SOT and PID group versus all other groups). For measles, having received SOT was inversely associated with seroprevalence (OR(95%CI): 0.28(0.08-1.0)). For mumps seroprevalence, non-significant trends were found for the number of doses (OR(95%CI): 2.5(1.0-6.7)) and time since vaccination (OR(95%CI): 0.89 (0.78-1.0)). For rubella seroprotection, a trend for time since vaccination was observed (OR(95%CI): 0.87(0.76-1.0)). Diphtheria and tetanus seroprotection were inversely associated with time since vaccination (diphtheria OR(95%CI): 0.80(0.71-0.89); tetanus OR(95%CI): 0.80 (0.68-0.93)). Anti-PT seropositivity was associated with number of received doses (OR(95%CI): 1.4(0.26-13.8)). Models for anti-PT titers ≥50IU/ml and ≥100IU/ml did not converge due to few observations in some groups. When omitting disease group as independent variable from models, the effect of time since last vaccination was statistically significant for seroprevalence against mumps (p=0.04) and seroprotection against rubella (p=0.01). Adding age or the presence of a relevant comborbidity as independent factors had a negligible effect on these models and no effect of these factors could be observed.

Table 3: Determinants of seroprotection rubella, diphtheria and tetanus and seroprevalence of measles, mumps and pertussis: multiple logistic regression

	Measles (≥150 mIU/ml)		**Mumps (≥230 labU/ml)**		**Rubella (≥10IU/ml)**		**Diphtheria (≥0.1IU/ml)**		**Tetanus (≥0.1IU/ml)**		**Pertussis (≥5IU/ml)**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**P**	**OR (95% CI)**	**p**	**OR (95% CI)**	**P**	**OR (95% CI)**	**p**
Number of doses	1.8 (0.65-5.5)	0.3	2.5 (1.0-6.7)°	0.052	1.9 (0.73-4.9)	0.2	1.2 (0.90-1.6)	0.2	1.2(0.71-2.0)	0.4	**1.4 (1.0-1.9)**	**0.03**
Time since last vaccination	0.95 (0.83-1.1)	0.5	0.89 (0.78-1.0)°	0.07	0.87 (0.76-1.0)°	0.050	**0.80 (0.71-0.89)**	**<0.001**	**0.80 (0.68-0.93)**	**0.006**	0.95 (0.86-1.0)	0.3
Disease group												
PID[a]	0.42 (0.14-1.7)	0.1	1.1(0.46-2.9)	0.8	1.35 (0.52-3.7)	0.5	0.72 (0.35-1.5)	0.4	0.38 (0.10-1.4)	0.1	0.63 (0.31-1.3)	0.2
SOT	0.28 **(0.08-1.0)**	**0.045**	0.82 (0.26-2.9)	0.7	0.60 (0.20-2.0)	0.4	0.75 (0.29-1.9)	0.5	1.4 (0.26-13.8)	0.7	0.55 (0.22 1.3)	0.2
Other patients	Reference		Reference		Reference		Reference		Reference		Reference	

[a] PID children who receive immunoglobulin G, were excluded from this analysis.

Discussion

This study provides new insights in the susceptibility of children with chronic illness to vaccine-preventable diseases. We found that up to 50% of chronically ill children remain vulnerable to vaccine-preventable diseases. Seroprevalence of antibodies was found in 83.3% for measles, in 77.9% for mumps and 80.6% was protected against rubella. Most patients were fairly well protected against tetanus, but only 61.3% against diphtheria and 53.2% had antibodies (≥5 IU/ml) against pertussis toxin. These rates are below those reported in the general Belgian population in 2006 and in other countries, such as the United States, Australia and Germany [9,11–14]. Heijstek et al. found similar seroprevalence rates for tetanus and rubella but higher titers for measles, mumps and diphtheria in children with juvenile idiopathic arthritis [24]. However, a direct comparison with seroprevalence studies in other populations is hampered by differences in vaccination policy, as well as variations in the disease epidemiology, choice of serological tests and seroprevalence cut-off values.

A first reason for the low seroprevalence is the low vaccination uptake. Only about 80% of the patients were age-appropriately vaccinated against MMR and 73% against DTP, which is far below the vaccination coverage in the general population of children and the WHO-target of 95% to eliminate measles and rubella [25,26]. The observed effects of age-appropriate vaccination, time since vaccination and number of received doses, however, provide clear evidence that vaccination is beneficial, even if a patient is chronically ill or has immunosuppressive conditions. For this reason, we advocate checking the vaccination status at each clinic visit. Reasons for lower vaccination coverage in children with chronic conditions are manifold. Firstly, vaccination is not always addressed during consultations with pediatricians due to time constraints. Secondly, these children might miss out on school-based vaccination due to frequent illness. Thirdly, parents might be somewhat reluctant to vaccination in these populations because of concerns about interactions with the chronic disease. This is why they do not always give consent to have vaccines given at school. In countries with a school-based program, school doctors might then think that the general practitioner or pediatrician will vaccinate the child, whereas these physicians believe the school doctor will follow-up on vaccination. Centralized vaccination registers accessible to all treating physicians should be used and consulted routinely during clinic visits to optimize the vaccination status.

Secondly, there are concerns that direct protection through vaccination may not be sufficient given the immunosuppressive characteristics of chronic disease or the use of immunosuppressive treatment [27]. This has been shown for diphtheria, tetanus, hepatitis B and influenza vaccination in pediatric and adult transplant patients [28]. Furthermore, Heijstek et. al. found lower seroprevalence rates for mumps, rubella, diphtheria and tetanus in children with juvenile idiopathic arthritis compared healthy age-matched controls [24]. In our study, we found lower seroprotection rates in transplant patients, except for tetanus. This is in line with Pedrazzi et al., who found maintenance of tetanus immunity but an accelerated loss of diphtheria antibodies after transplantation [29].

Children with a solid-organ transplant were found to be particularly vulnerable. Seroprevalence of measles was significantly lower in SOT patients compared to the other groups, but that of other vaccine-preventable diseases was also below the desired level. Given the contra-indication of live-attenuated vaccines and the recommendation to postpone all other vaccines until six to twelve months after transplantation,[3] it is hence important to follow the recommendation by the Belgian NITAG to evaluate and complete the vaccination schedule before transplantation [3]. The contra-indication for live-attenuated vaccines also applies to PID patients, but the MMR seroprevalence was relatively high in this group. This is probably in part due to the IgG-treatment that is given, but the uptake of MMR vaccination was also higher. Many PID children had received the first dose scheduled at 12 months of age, because the majority was diagnosed after that age. Only few children with PID were above 11 years of age, and thus required a second dose of MMR for age-appropriate vaccination.

Seroprevalence thresholds for herd immunity are estimated to be 92-95% for measles and pertussis, 90-92% for mumps, 85-87% for rubella and 80-85% for diphtheria. These rates are not reached in all our patient groups, and neither in all age-groups in the community [9,30]. Seroprevalence below desired levels has been related to a decrease in vaccination coverage, delay in vaccination, primary vaccine failure and waning immunity and can lead to outbreaks. Resurging cases of measles, mumps and pertussis in the past decade indicate that herd protection is inadequate, and that a relatively large number of vulnerable children with chronic disease may be at risk. Due to the lack of recent data on seroprevalence it is not clear if these children benefit from community immunity.

A strength of this study is that it assesses seroprevalence in a broad range of at-risk children and that it directly relates seroprevalence of antibodies with vaccination status. Nonetheless, some limitations should be addressed. A first limitation is the absence of a control group. Secondly, the number of children in some groups were too small for a comparison with the other disease groups. Thirdly, the study was performed in a tertiary care hospital which curbs extrapolation of results to all at-risk patients. Fourthly, it is unknown whether antibodies for mumps and pertussis were derived from vaccination or from natural infection as there was a large mumps outbreak in 2013-2014 in Flanders and pertussis cases have been increasing since 2011. For measles, on the contrary, we expect that antibodies are only derived from vaccination as measles only reemerged in Belgium after the survey had ended. Another limitation of this study is that only ELISA has been used for the assessment of mumps antibodies. Whereas ELISA measures all mumps antibodies, seroneutralization tests only measure functional antibodies. Lastly, there was a potential underreporting of vaccination, which is indicated by high levels of seroprotection in unvaccinated patients against diseases that are not endemic, such as rubella.

We conclude that children with chronic diseases are at particular risk for vaccine-preventable diseases. This suggests a two-fold flaw in the current vaccination programs. Firstly, children with chronic diseases are less vaccinated compared to healthy children and secondly, vaccines might provide less protection than expected in this population. This is especially true for measles, mumps and pertussis, vaccine-preventable diseases that have been resurging in the last decade. At present it is unclear whether the degree of

protection in the community is sufficient to protect these patients. As vaccination positively contributes to protection through induction of antibodies, it is imperative to follow vaccination status of chronically ill children more closely and to pay more attention to pre-transplant vaccination of children who are candidates for SOT.

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CHAPTER 7: Attitudes, beliefs, determinants and organizational barriers behind the low seasonal influenza vaccination uptake in healthcare workers – A cross-sectional survey

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Abstract

Background

Seasonal influenza threatens hospitalized patients and residents of nursing homes annually. Due to age and chronic disease their protection following immunization is diminished. Additional immunization of direct contacts and in particular healthcare workers (HCWs) has proven added value. As vaccination coverage in HCWs remains low, we aimed to gain insight in the factors behind the demotivation for influenza vaccination.

Methods

Attitudes and believes towards influenza vaccination and socio-demographic and professional determinants were surveyed in 5141 Belgian HCWs from 13 hospitals and 14 nursing homes. Additionally, influenza campaign coordinators of the participating healthcare institutions were interviewed about the factors of success/failure in their campaigns.

Results

The mean vaccination coverage registered by the participating healthcare institutions was 40.4% in the hospitals and 45.3% in the nursing homes. Overall, up to 90% of HCWs found it important not to infect their patients. However, only 20% of non-vaccinated HCWs considered influenza vaccination a duty to not harm their patients. Up to 40% of unvaccinated staff believed they could get influenza after vaccination and that vaccination weakens their immune system. Also, only about 20% of unvaccinated staff thought to have a high chance of getting influenza. Reasons for unvaccinated staff to get vaccinated in the future are self-protection and protection of family members. Factors that positively influenced vaccination coverage are encouragement by supervisors (OR, hospitals: 7.1, $p<0.001$; nursing homes: 7.5, $p<0.001$) and well-organized vaccination campaigns with on-site vaccination. Factors that negatively affected vaccination coverage are misconceptions about influenza and its vaccine (OR, range 0.1-0.7, $p<0.001$ for most misconceptions) and underestimation of the risk of contracting influenza by patients or HCWs (OR of perceived susceptibility, range 2.1-5.1, $p<0.001$ for most factors).

Conclusion: There is a need for guidance for the organization of seasonal influenza campaigns, in which education, communication and easy-accessible vaccination are promoted.

Keywords: Healthcare workers, influenza, vaccination, motivation, barriers

Introduction

Seasonal influenza is an infectious disease that threatens public health every year. A recent study estimated that 291,000 to 646,000 individuals die from seasonal influenza associated respiratory complications annually [1]. Up to 90% of these lethal cases occur in the age group ≥65 [2]. Immunocompromised patients and persons with a chronic disease have a 4- to 10-fold increased risk of hospitalization and complications caused by influenza [3,4]. Since direct protection of these patients through vaccination is diminished, it needs to be supplemented with vaccination of their direct contacts, in particular healthcare workers (HCWs). The latter is of high importance since as many as 25% of HCWs get infected with influenza annually and may thus transmit influenza to patients prior to having symptoms. Moreover, it has been shown that vaccinating HCW is an effective strategy in reducing all-cause mortality and influenza-like illness in patients and residents of healthcare institutions (HCIs) [5-9]. To this end, influenza vaccination for all HCWs has been recommended. Despite the known advantages of immunizing HCWs, coverage rates are generally low, and range from 14% in Poland to 45.6% in England according to a recent review [10]. Reasons for low influenza vaccination uptake are diverse and comprise organizational barriers, such as lack in time and poor accessibility, doubts about the effectiveness, fear for side effects and personal reasons including the right to become ill themselves [11-19].

However, most studies tended to focus on HCWs from hospitals or nursing homes, rather than focusing on HCWs from both types of HCI. Furthermore, only limited studies have focused on the composite of demographic, behavioral and organizational factors that are associated with vaccination uptake [13,14]. Insights in these factors are important for the development of specific influenza vaccination programs that aim to increase vaccination coverage. For this reasons, we aimed to determine demographic, behavioral and organizational factors that are associated with vaccination uptake in HCWs in both hospitals and nursing homes in Flanders, Belgium.

Methods

Study population and procedure

In October 2015, 22 hospitals and 47 nursing homes of different size were approached for study participation in Flanders, Belgium. Of those HCIs, 13 hospitals and 14 nursing homes agreed to participate. An anonymous online survey was used to determine social, demographic and behavioral factors and beliefs that were associated with influenza vaccination uptake among HCWs. A link to the online survey was provided to all staff of the participating HCIs, further defined as HCWs, in November 2015, and was available for 6 weeks. A reminder was sent twice to all HCWs and promotional material (posters and tissue boxes) was distributed in the HCIs to promote participation. Participation in the survey was voluntary and anonymous. Only fully completed questionnaires were used for data analysis. In addition, possible key factors of success/failure during the organization of influenza vaccination campaigns were identified with semi-structured interviews with the organizers of influenza vaccination campaigns in the participating HCIs. The study was approved by the Ethical Committee of the University Hospitals of Leuven, Belgium (S58512).

Online survey (HCW)

The online survey was designed with LimeSurvey (LimeSurvey Project, Hamburg, Germany) and based on questionnaires that were previously used in the Netherlands to ensure comparability [13,14]. The survey was divided into four parts: (i) demographics; (ii) knowledge about the recommendations of the Superior Health Council; (iii) the perception of influenza and the influenza vaccine; and (iv) behavior. The behavioral part was based on the Health Belief model, the Health Intention Model and the attitude/social influence/self-efficacy (ASE) model [20-22]. Finally, depending on the vaccination status in the previous year (2014), respondents answered 5 or 6 additional questions about reasons for or against vaccination. The complete questionnaire is added in supplementary (currently only in Dutch).

In-depth semi-structured interviews (HCI)

In person semi-structured interviews were conducted with the influenza campaign organizers of the participating HCIs and took approximately one hour. The interviews contained items on the demographic profile of the institution, the vaccination coverage in the current (2015) and previous two seasons, and open-ended questions on the methods that were used for organizing the campaign, and actions that had been taken to increase the vaccination coverage. All interviews were written out and compared. In case of ambiguity, the organizers were contacted once more. In order to identify factors leading to success, methods used in HCIs with a high vaccination coverage were analyzed separately. The structure of the interview is added in supplementary (currently only in Dutch).

Statistical analysis

A sample size of 500 participants per province (n_{total}=2500) for HCWs in hospitals was calculated based on an estimated vaccination coverage of 50% and a 95 % confidence interval (CI) [11]. Since the staffing levels in nursing homes are generally lower, a sample size of 500 HCWs over all five provinces was targeted for recruitment. Questions on the 5-point Likert scales were dichotomized analogously to previous surveys by combining (i) “Strongly agree” and “agree” as a positive response and (ii) “do not agree/do not disagree”, “disagree” and “strongly disagree” as a negative response [13,14]. Univariate and multivariate logistic regression analysis and chi-squared tests were used to identify factors (demography, beliefs, behavior) that influence influenza vaccination coverage in HCWs. Results are expressed as odds ratios (OR) and their 95% confidence intervals (CI). In the multiple regression analysis, final models were selected by backwards elimination of non-significant variables, using the Mallow Cp criterion [23]. A test probability of 5% was considered statistically significant. All data were analyzed with R. version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, 2013).

Results

Participation and characteristics of healthcare institutions and participants

In total, 28 790 HCWs, of which 26 524 were hospital staff and 2 266 nursing home staff, received a link to complete the survey. The total response rate among HCWs was 17.9%: 17.0% in the hospitals and 27.9% in the nursing homes. The respondents were mainly women (79% in hospitals and 88.5% in nursing homes) and the mean age was 42.6 years in the hospitals and 43.5 years in the nursing homes. All demographic characteristics of our study population are listed in table 1 and are comparable to available census data on Flemish HCWs. The vaccination coverage of all HCWs in the participating HCIs (as registered by the HCIs) from season 2013-2014 until season 2015-2016 is represented in figure 1. The mean vaccination coverage registered by the participating HCIs was 40.4% in the hospitals and 45.3% in nursing homes during the last influenza season (2015-2016). Based on self-reported vaccination status of 2014, 62.4% and 52.6% of the respondents of respectively hospital and nursing homes claimed to be vaccinated.

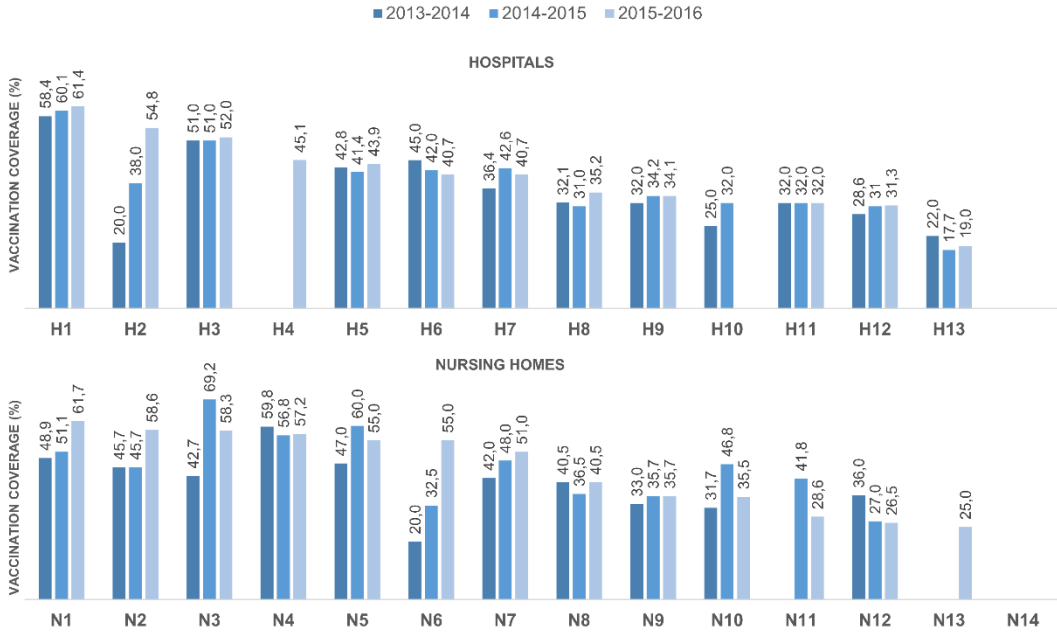

Figure 1: Vaccination coverage in participating hospitals and nursing homes from seasons 2013-2014 to 2015-2016, in descending order according to the coverage in 2015-2016. H1 to H13 and N1 to N14, respectively, hospitals and nursing homes ranked according to vaccination coverage, with H1 and N1 being the hospital and nursing home with the highest vaccination coverage.

Table 1: Characteristics and vaccination status of participants

	Hospitals (N = 4506)	Nursing homes (N = 635)
Personal data	**n (%)**	**n (%)**
Female gender ***	3561 (79.0)	562 (88.5)
Mean age, years (SD) ***	42.6 (11.5)	43.5 (11.3)
Marital state ***		
Married/cohabiting	3545 (78.7)	482 (75.9)
Single	219 (4.9)	60 (9.4)
Divorced	742 (16.5)	93 (14.6)
Having children at home	2643 (58.7)	363 (57.2)
Chronic illness	251 (5.6)	38 (6.0)
Education ***		
Master degree	1240 (27.5)	34 (5.3)
Bachelor degree	2469 (54.8)	151 (23.8)
Secondary education or lower	797 (17.7)	450 (70.9)
Professional data	**n (%)**	**n (%)**
Occupation		
Physician	558 (12.4)	1 (0.2)
Nurse	1759 (39.0)	60 (9.4)
Nursing assistant	385 (8.5)	103 (16.2)
Midwife	137 (3.0)	NA
Nursing Aides	140 (3.1)	240 (37.8)
Other HCWs †	750 (16.6)	71 (11.2)
Administrative, facilities and logistics	773 (17.2)	160 (25.2)
Years of work in healthcare sector		
< 5 years	795 (17.6)	121 (19.1)
5-9 years	787 (17.5)	119 (18.7)
10-20 years	1051 (23.3)	157 (24.7)
20-30 years	950 (21.1)	146 (23)
30-40 years	843 (18.7)	88 (13.9)
> 40 years	80 (1.8)	4 (0.6)
Work situation		
Full time	2726 (60.5)	315 (49.6)
Part time	1780 (39.5)	320 (50.4)
Daily contact with patients	3472 (77.1)	565 (89.0)
Vaccination status study population	**n (%)**	**n (%)**
Vaccinated in 2014	2822 (62.6)	334 (52.6)
Vaccinated in 2015	2918 (64.8)	355 (55.9)
Never vaccinated	753 (16.7)	135 (21.3)

***P (<0.001) for difference between hospitals and nursing homes
† Medical technical staff, pharmacists, audiologists, physiotherapists, paramedics, psychologists, researchers
HCW: Healthcare worker

Determinants of vaccination

Demographical determinants

Demographic factors that are univariately associated with vaccination uptake in hospitals or nursing homes are listed in table 2. Male gender, older age, chronic illness, higher education and working irregular shifts or night shifts only were significantly associated with influenza vaccination uptake. In hospitals, physicians were significantly more likely to be vaccinated than nurses, whereas midwives, nursing assistants and nursing aides were less likely to be vaccinated. In nursing homes, nurses were significantly more likely to be vaccinated than nursing assistants and nursing aides.

Behavioral determinants: Perception about influenza and influenza vaccination

Nearly all behavioral determinants were univariately associated with vaccination uptake (table 3). HCWs were more likely to take up influenza vaccination if it was encouraged by their supervisor or by their close contacts. In case of attitude specific determinants associated with a higher vaccination uptake were 'finding it important not to infect patients' and 'considering vaccination as a duty not to harm patients'. Also 70-90% of HCWs found it important to have the freedom to decide whether to take the vaccine or not. HCWs who have confidence that the vaccine protects themselves and their patients were more likely to be vaccinated. In the domain of 'perceived barriers' unvaccinated HCWs significantly agree more with barrier statements. For example, as much as 37.6% of unvaccinated hospital staff and 29.1% of unvaccinated nursing home staff believed that influenza is not dangerous. Similarly, unvaccinated HCWs believed that vaccination weakens the immune system (29.7% in hospitals and 42.5% in nursing homes) and that you can get influenza from the vaccine (36.7% in hospitals and 42.9% in nursing homes). Concerning perceived susceptibility 'believing to have a high chance of getting influenza' and 'believing to have a high chance of infecting patients' influences influenza vaccination positively.

Multivariate analysis

Since little differences between hospitals and nursing homes were observed in the univariate analysis, data were pooled for the multivariate analysis. When including type of HCI (hospital versus nursing home) as an additional variable, no difference in final multivariate model could be observed (not shown). Age, education, work experience and chronic illness are the personal characteristics that remain statistically significant after backward elimination of other factors in the multivariate analysis. 'Confidence that the vaccine will protect against influenza' and 'considering influenza vaccination a duty in order not to harm patients' were among other behavioral determinants associated with vaccine uptake (table 4)

Table 2: **Distribution of demographic characteristics (%) according to vaccination uptake in HCW (univariate logistic regression)**

	Hospitals (N = 4506)			**Nursing homes** (N = 635)		
Vaccinated:	No	Yes	OR (95% CI)	No	Yes	OR (95% CI)
N:	1684	2822		301	334	
Personal data						
Gender (vs. female)						
Male	**17.0**	**23.3**	**1.5 (1.3 – 1.7) *****	**7.3**	**5.3**	**2.3 (1.4 – 3.9) ****
Age						
≥ 25 years (vs < 25 years)	**89.3**	**96.6**	**3.4 (2.7 – 4.4) *****	90.4	93.4	1.5 (0.9 – 2.7)
≥ 50 years (vs < 50 years)	**24.4**	**34.6**	**1.6 (1.4 – 1.9) *****	**29.2**	**40.1**	**1.6 (1.2 – 2.3) ****
Marital state (vs single or divorced)						
Married or cohabiting	**75.2**	**80.7**	**1.4 (1.2 – 1.6) *****	75.7	76.0	1.0 (0.7 – 1.5)
Children living at home	**54.8**	**60.9**	**1.3 (1.1 – 1.5) *****	59.1	55.4	0.9 (0.6 – 1.2)
Chronic illness	**2.6**	**7.3**	**3.0 (2.1 – 4.2) *****	**2.0**	**9.6**	**5.2 (2.3 – 14.0) *****
Education						
Master degree	**20.4**	**31.8**	**1.6 (1.4 – 1.9) *****	4.7	6.0	0.8 (0.4 – 1.8)
Bachelor degree	56.7	53.6	reference	18.3	28.7	reference
Secondary education or lower	**22.9**	**14.6**	**0.7 (0.6 – 0.8)*****	**77.1**	**65.3**	**0.5 (0.4 – 0.8) *****
Professional data						
Occupation[2]						
Physician	**5.9**	**16.2**	**2.6 (2.1 – 3.4) *****	NA	NA	NA
Nurse	**38.4**	**39.4**	reference	6.0	12.6	reference
Midwife	**4.3**	**2.3**	**0.5 (0.4 – 0.7) *****	NA	NA	NA
Nursing assistant	**11.9**	**6.6**	**0.5 (0.4 – 0.7) *****	**16.3**	**16.2**	**0.5 (0.2 – 0.9) ***
Nursing aide	**4.0**	**2.6**	**0.6 (0.4 – 0.9) *****	**44.5**	**31.7**	**0.3 (0.2 – 0.6) *****
Other HCWs [1]	17.3	16.3	0.9 (0.8 – 1.1)	**11.3**	**11.1**	**0.5 (0.2 – 1.0) ***
Administrative, facilities, logistics	18.2	16.7	0.9 (0.8 – 1.1)	21.9	28.1	0.6 (0.3 – 1.1)
Years of work in healthcare sector[3]						
< 5 years	**24.6**	**13.5**	**0.5 0.4 – 0.6) *****	21.6	16.8	0.7 (0.5 – 1.2)
5-9 years	18.1	17.1	0.9 (0.8 – 1.1)	18.6	18.9	1.0 (0.6 – 1.6)
10-19 years	23.0	23.5	reference	24.3	25.1	reference
20-29 years	**18.3**	**22.7**	**1.2 (1.0 – 1.5) ***	21.9	24.0	1.1 (0.7 – 1.7)
30-39 years	**15.0**	**20.9**	**1.4 (1.1 – 1.7) ****	13.3	14.4	1.0 (0.6 – 1.8)
> 40 years	**1.0**	**2.2**	**2.2 (1.3 – 3.9) ****	0.3	0.9	2.6 (0.3 – 53.3)
Schedule						
Day shifts only	**43.9**	**51.7**	reference	33.9	44.9	reference
Irregular shifts	**52.0**	**45.0**	**0.7 (0.6 – 0.8) *****	**58.8**	**50.6**	**0.6 (0.5 – 0.9) ****
Only night shifts	**4.2**	**3.4**	**0.7 (0.5 – 1.0) ***	**7.3**	**4.5**	**0.5 (0.2 – 0.9) ***
Daily contact with patients	76.9	77.1	1.0 (0.9 – 1.2)	89.7	88.3	0.9 (0.5 – 1.4)

* p < 0.05, ** p < 0.01, *** p < 0.001 (univariate logistic regression)
[1] Other Health Care Workers: medical technical staff, pharmacists, audiologists, physiotherapists, paramedics, psychologists, researchers
NA: not applicable; OR: Odds Ratio, CI: confidence interval

Table 3: Behavioral characteristics according to vaccination uptake in HCW (univariate logistic regression)

	Hospitals N = 4506			Nursing homes (N = 635)		
Vaccinated:	No	Yes	OR (95 % CI)	No	Yes	OR (95 % CI)
N:	1684	2822		301	334	
Social influences						
People close to me find it important that I get vaccinated against influenza	7.1%	36.1	7.4 (6.0 – 9.0) ***	7.6	41.6	8.6 (5.4 – 14.2) ***
My colleagues find it important that I get vaccinated against influenza.	8.6	25.7	3.7 (3.0 – 4.5) ***	6.6	22.8	4.2 (2.5 – 7.1) ***
The head of my ward should recommend influenza vaccination	22.3	67.1	7.1 (6.2 – 8.2) ***	22.9	69.2	7.5 (5.3 – 10.8) ***
I find it important to follow the advice of the people close to me.	16.6	39.0	3.2 (2.8 – 3.7) ***	17.6	49.7	4.6 (3.2 – 6.7) ***
Self-efficacy						
I would definitely take up influenza vaccination if:						
• The vaccine was given at convenient time	17.8	70.4	11.0 (9.4 – 12.8) ***	16.6	63.5	8.7 (6.0 – 12.8) ***
• The vaccine was given at my ward.	19.1	73.8	11.9 (10.3 – 13.9) ***	16.9	73.4	13.5 (9.2 – 20.0) ***
• It was rewarded.	11.2	27.7	3.1 (2.6 – 3.6) ***	12.0	26.0	2.6 (1.7 – 4.0) ***
• I received a reminder.	13.6	51.4	6.7 (5.8 – 7.9) ***	11.3	47.0	7.0 (4.6 – 10.7) ***
Attitude						
I find it important that:						
• HCWs do not infect patients.	87.1	96.7	4.4 (3.4 – 5.6) ***	80.4	95.5	5.2 (2.9 – 9.7) ***
• All HCWs are vaccinated against influenza to ensure continuity of care.	26.1	76.0	9.0 (7.8 – 10.3) ***	24.3	75.4	9.6 (6.7 – 13.9)***
• All nurses are vaccinated against influenza	24.1	77.3	10.7 (9.3 –12.3) ***	18.6	73.7	12.2 (8.4 – 18.0) ***
• HCWs have the freedom to decide whether or not to have the influenza vaccine	91	70.1	0.2 (0.2 – 0.3) ***	85.0	71.0	0.4 (0.3 – 0.6) ***
• Only vaccinated HCWs are allowed to work during influenza epidemics.	2.8	4.7	1.7 (1.2 – 2.4) **	6.6	5.7	0.8 (0.4 – 1.6)
• Unvaccinated HCWs cannot work and should not receive salary during influenza epidemics	1.5	5.0	3.4 (2.2 – 5.27) ***	2.7	5.7	2.2 (1.0 – 5.4)
I consider influenza vaccination important as it is my duty not to harm patients	**21.4**	**74.1**	**10.5 (9.1 – 12.1) *****	**17.9**	**69.8**	**10.6 (7.3 – 15.5) *****
I think influenza vaccination should be mandatory for HCWs	6.9	33.0	6.7 (5.4 – 8.2) ***	7.3	41.9	9.2 (5.7 – 15.2) ***
I am planning to have the influenza vaccine next year	19.3	93.1	56.6 (47.0 – 68.6) ***	16.3	90.1	46.9 (29.7 – 76.4) ***
Advantages of influenza vaccination						
Vaccination against the flu gives me confidence that:						
• I will not get influenza	23.9	69.4	7.2 (6.3 – 8.3) ***	15.3	63.2	9.5 (6.5 – 14.1) ***
• I will not infect patients	31.1	75.8	6.9 (6.1 – 8.0) ***	28.6	71.6	6.3 (4.5 – 8.9) ***
• I will not infect my family	30.4	76.9	7.6 (6.7 – 8.8) ***	27.9	69.5	5.9 (4.2 – 8.3) ***
I find it important that all HCWs are vaccinated in order to avoid increased workload	17.3	71.8	12.1 (10.5 – 14.1) ***	22.3	73.1	9.5 (6.6 – 13.7) ***
Perceived barriers against vaccination	-	+	OR	-	+	OR
I think influenza is not dangerous to me	37.6	24.4	0.5 (0.5 – 0.6) ***	29.9	21.9	0.7 (0.5 – 0.9) *
Vaccination weakens my immune system	29.7	7.4	0.2 (0.2 – 0.2) ***	42.5	15.6	0.2 (0.2 – 0.4) ***
I can get the flu because of the vaccine	36.7	15.9	0.3 (0.3 – 0.4) ***	42.9	21.9	0.4 (0.3 – 0.5) ***
I am generally against vaccination	15.7	2.4	0.1 (0.1 – 0.2) ***	29.6	3.9	0.1 (0.1 – 0.2) ***
I am especially against vaccination in HCWs	8.1	1.2	0.1 (0.1 – 0.2) ***	12.3	2.1	0.2 (0.1 – 0.3) ***
I think HCI only offer influenza vaccination to reduce costs	8.7	5.2	0.6 (0.5 – 0.7) ***	10.6	6.9	0.6 (0.4 – 1.1)
If take up influenza vaccination once, I have to do this every year	18.5	22.5	1.3 (1.1 – 1.5)**	21.6	25.7	1.3 (0.9 – 1.8)
Perceived susceptibility to influenza						
I think I have a high chance of getting influenza	18	51.1	4.8 (4.1 – 5.5) ***	19.9	47.3	3.6 (2.5 – 5.2) ***
I think influenza is very dangerous for my patients	58.8	77.8	2.5 (2.2 – 2.8) ***	82.4	90.7	2.1 (1.3 – 3.4) **
I think I have a high chance to infect patients	38.7	76.2	5.1 (4.5 – 5.8) ***	52.2	83.8	4.8 (3.3 – 6.9) ***
I think HCWS have an increased risk of getting ill during an influenza epidemic.	61.6	84.5	3.4 (3.0 – 3.9) ***	62.5	86.5	3.9 (2.6 – 5.8) ***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

CI confidence interval; HCI: Healthcare institution; HCW: Healthcare worker; OR: Odds ratio.

Table 4: Demographic and behavioral characteristics associated with vaccination uptake in HCWs (multiple logistic regression)

Determinants	OR (95 % CI)
Socio-demographic factors	
Age (vs. ≤ 25 years)	
25-50 years	**2.1 (1.4 – 3.1) *****
50-60 years	**2.2 (1.3 – 3.6) ****
>60 years	**2.6 (1.2 – 5.8) ***
Education (vs. bachelor degree)	
Master degree	0.9 (0.7 – 1.1)
Secondary education or lower	**0.6 (0.5 – 0.8) *****
Years of work in healthcare sector (vs. 10-19 years)	
<5 years	**0.5 (0.4 – 0.7) *****
5- 9 years	1.0 (0.8 – 1.4)
20-29 years	1.1 (0.8 – 1.5)
30-39 years	1.0 (0.6 – 1.4)
≥ 40 years	1.2 (0.5 – 3.2)
Absence of chronic illness	**0.5 (0.3 – 0.8) ****
Social influences	
People close to me find it important that I get vaccinated against influenza	1.2 (1-1.6)
My colleagues find it important that I get vaccinated against influenza.	0.8 (0.6-1.1)
Self-efficacy	
I would definitely take up influenza vaccination if the vaccine was given at my ward.	**1.4 (1.1 – 1.8) ****
Attitude: I think it is important that:	
All HCWs are vaccinated against influenza to ensure continuity of care.	1.2 (0.9 – 1.5)
All nurses are vaccinated against influenza	1.3 (1 – 1.6)
HCWs have the freedom to decide whether or not to have the influenza vaccine	**0.5 (0.4 – 0.7) *****
Only vaccinated HCWs are allowed to work during influenza epidemics.	**0.6 (0.4 – 1.0) ***
Unvaccinated HCWs cannot work and should not receive salary during influenza epidemics	1.7 (1.0 – 3.1)
I consider influenza vaccination important as it is my duty not to harm patients	**1.5 (1.2 – 1.9) *****
I am planning to have the influenza vaccine next year	**16.7 (12.9 – 21.6) *****
Advantages of influenza vaccination	
Vaccination against the flu gives me confidence that I will not get influenza	**1.4 (1.1 – 1.7) ****
Perceived barriers against vaccination	
I think influenza is not dangerous to me	**0.8 (0.7 – 1.0) ***
Vaccination weakens my immune system	0.8 (0.6 – 1.1)
I can get the flu because of the vaccine	**0.8 (0.6 – 1.0)***
I am generally against vaccination	**0.6 (0.4 – 0.9) ***
I am especially against vaccination in HCWs	**0.5 (0.3 – 0.8) ****
I think HCI only offer influenza vaccination to reduce costs	1.4 (1.0 – 2.0)
If take up influenza vaccination once, I have to do this every year	0.8 (0.7 – 1.1)
Perceived susceptibility to influenza	
I think I have a high chance of getting the flu	**1.7 (1.4 – 2.1) *****
I think I have a high chance to infect patients	**1.5 (1.3 – 1.9) *****
I think HCWS have an increased risk of getting ill during an influenza epidemic.	**0.7 (0.6 – 0.9) ****

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
HCW: Healthcare worker; OR: Odds ratio, CI: confidence interval

Reasons for vaccination and non-vaccination

Vaccinated HCWs reported protection of patients against influenza (76.7% in hospitals and 71.9% in nursing homes), their own protection (74.0% in hospitals and 72.8% in nursing homes) and the wish to protect family members (64.0% in hospitals and 53.9% in nursing homes) as the most important motivators for vaccination. Among unvaccinated staff, not being convinced about the efficacy of the vaccine (43.1% in hospitals 55.8% in nursing homes), doubts about the usefulness of the vaccine (28.5% in hospitals and 32.9% in nursing homes) or the necessity of annual vaccination (22.1% in hospitals and 20.3% in nursing homes) were the most important reasons for non-vaccination. Reasons that may convince unvaccinated HCWs to have influenza vaccination in the future are having an increased risk for complications themselves (45.5% in hospitals and 36.9% in nursing homes) and having a family member at increased risk of getting influenza (39.3% in hospitals and 25.6% in nursing homes).

Organizational factors of success/failure: results from in-depth semi-structured interviews

Thirty-five persons responsible for seasonal influenza campaigns (at least one of each participating HCI) were interviewed. Twenty-two were women and the mean age was 42.3 years (range: 27-63 years of age). Low-threshold vaccination was identified as a most important factor of success. Five out of the seven hospitals with an above average vaccination coverage, vaccinated their HCWs on the ward. In addition, the three best vaccinating hospitals used a mobile cart program, and the two best vaccinating hospitals did not require advance registration. Further, efficient communication was given high priority in HCIs with a high vaccination coverage. Program organizers stressed the need to repeat messages through multiple communication channels. Also personalized communication, including information with colleagues of the HCI promoting vaccination, was raised as possible solution to increase coverage rates. Another key factor of success is education. All hospitals and all nursing homes, except for one, agreed that information and explanation about influenza and the influenza vaccine might be helpful to increase vaccination coverage. In addition, the involvement of supervisors in the influenza vaccination campaign was considered important. Lastly, the use of a reward was generally seen as a potential incentive for vaccination, and one hospital (the best vaccinating) and one nursing home (with average coverage rate) did effectively reward its staff after vaccination. Finally, organizers of the influenza vaccination campaigns generally believe that misconceptions about influenza and the influenza vaccine are the main issue to address.

Discussion

To the best of our knowledge, this is one of the largest studies that has been conducted to assess the motivation of HCWs towards influenza vaccination [24-26]. Moreover, HCWs from both hospitals and nursing homes were included, which allows for a direct comparison of HWCs from both types of HCIs. Since the profile of HCWs differs between hospitals and nursing homes, and since both offer a different type of care (i.e. acute treatment in hospitals versus long-term residential care in nursing homes) to different populations, it is useful to consider motivators for vaccination in both types of healthcare settings. Another strength of this study is that next to demographic and behavioral determinants, organizational factors of influenza vaccination were considered, which makes it unlikely that we missed potential motivators.

A remarkable result is that up to 90% of HCWs found it important not to infect their patients but only 20% of non-vaccinated HCWs and 70% of vaccinated HCWs considered influenza vaccination a duty in order not to harm the patients they care for. A possible explanation is disbelief in the efficacy and the usefulness of the vaccine [27,28]. Such disbelief can be fueled by the difficulty to distinguish influenza from other influenza-like illnesses. A recent study found that influenza vaccination did not decrease the incidence of influenza-like illness (ILI) because the virus was replaced by other respiratory pathogens as measured through a PCR-based multiplex ligation-dependent probe amplification (MLPA) assay [29]. To the public ILIs are usually synonymous for influenza, although they can be caused by many other respiratory pathogens. Since the incidence of ILI does not decrease with influenza vaccination, even though influenza does, it gives the impression that the flu vaccine is not efficient and should therefore not be used. In this context, we believe that large scale randomized controlled trials to prove the efficacy of HCW vaccination on laboratory confirmed influenza in patients, would be more helpful than studies on ILI alone.

Our findings on demographic factors associated with vaccination are largely in agreement with those from previous studies, such as age [13,14,16,30-32], male gender [13,14, 30-32], chronical illness [13,14,31], years of work in the healthcare sector [13,14], and higher education [14,32].

In line with the results of Hopman and Looijmans and colleagues, nearly all behavioral determinants were significantly associated with vaccination uptake [13,14]. Accordingly, we also found that misconceptions about influenza and its vaccine are still circulating and more frequently among unvaccinated HCWs. Moreover, the risk of contracting influenza and transmitting it to their patients was highly underestimated. All of this, calls for better education of HCWs and emphasizes the importance of awareness about the risk of influenza.

Reasons that would motivate unvaccinated staff to take up influenza vaccination in the future were self-protection and protection of family members. This shows that it is important to not only focusing on the value for the patients during influenza vaccination campaigns, but also on the personal benefits for the HCWs themselves [33,34]. Furthermore, the majority of HCWs also found it important to have the freedom to decide whether to take up influenza vaccination or not. Since influenza vaccination coverage among HCWs remains low, it has been questioned whether the right of freedom surpasses the duty to not harm patients. As a result, some advocate mandatory influenza vaccination for HCW [35,36]. In the USA, some HCIs have already implemented this strategy and attained a vaccination rate of >90% [37]. In our study, only a minority of HCW agreed that influenza vaccination should be mandatory. As long as the debate about mandatory vaccination is ongoing, the vaccination coverage can only be increased by providing guidance for the organization of seasonal influenza campaigns. During such campaigns, possible success factors that should be promoted according to the results of our study are education, communication, easy-accessible vaccination and involvement of supervisors. Additionally, the determinants that were associated with vaccination uptake can be used to fine-tune the objectives of the campaign and to determine the best strategy. For example, perceived barriers might be reduced through a proper education plan that includes information sessions and the use of informative flyers and posters. Another example is that social influences

can be addressed through the use of personalized promotional material in which known HCWs of a particular HCI are the key persons. Demographic determinants such as gender, age and occupation can be used to define specific subgroups for intervention. Studies have shown that such well-prepared campaigns can be effective to increase vaccination uptake [38-40]. To achieve this in Flanders, Belgium, and in follow-up of the current study, an instruction manual, scientific brochure and promotional materials were developed for the implementation of performant and successful vaccination campaigns in all Flemish hospitals and nursing homes (available in Dutch on: http://www.laatjevaccineren.be/hou-griep-uit-je-team). Future work will determine whether the use of this manual and related documents can improve vaccination coverage.

A first limitation of the study was that it was executed simultaneously with the influenza vaccination campaign and some HCWs might not have had the possibility to receive the vaccine, therefore self-reported vaccination state from the previous year (2014) was taken into account. Possible recall bias cannot be excluded. However, it is unlikely that an HCW who has never taken up influenza vaccination consequently ended up in the wrong response group. Secondly, participation in the study was performed on a voluntary base. It is possible that motivated (and vaccinated) HCWs were more likely to participate and to complete the survey than their unvaccinated peers. This response bias can be estimated from the slightly higher vaccination coverage among the respondents compared to the mean overall vaccination coverage of the participating HCIs. Nevertheless, about 36.3% of the responding HCWs in our survey was not vaccinated in 2015 and 19.1% was never vaccinated. This reassures us that the data are robust and can also give proper insight in attitudes and beliefs of non-vaccinated HCWs. Lastly, the response rate was relatively low (17.9%), which could lead to a non-response bias. However, demographic characteristics of respondents were comparable to census data on Flemish HCWs and separate analysis on determinants of vaccination for each participating HCI (data not shown) gave similar results in institutions with a low or high vaccination coverage. This supports that our study sample is representative for Flemish HCWs.

In summary, factors that positively influence vaccination coverage are encouragement by a supervisor, low-threshold vaccination and proper education and communication. These findings hold promise for the refinement of the current influenza vaccination programs.

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CHAPTER 8: Increased vaccine uptake and less perceived barriers towards vaccination in long-term care facilities that use multi-intervention manual for influenza campaigns

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Abstract

Seasonal influenza is an annually recurring threat to residents of long-term care facilities (LTCFs) since high age and chronic disease diminish immune response following vaccination. Although immunization of healthcare workers (HCWs) has proven to be an added value, coverage rates remain low. A ready-to-use instruction manual was designed to facilitate implementation of interventions known to increase vaccination coverage in healthcare institutions. It includes easy-access vaccination, role model involvement, personalized promotional material, education and extensive communication. We evaluated this manual during the 2017-vaccination campaign in 11 LTCFs in Belgium. Vaccination coverage before and after the campaign was recorded by the LTCFs and the usefulness of the manual was assessed by interviewing the organizers of the local campaigns. Attitudes towards vaccination and reasons for vaccination were evaluated with a quantitative survey in HCWs before and after the campaign. The mean vaccination coverage reported by the LTCFs was 54% (range: 35-72%) in 2016 and 68% (range: 45-81%) in 2017. After the campaign, HCWs were less likely to expect side effects after influenza vaccination (OR(95%CI): 0.4(0.2-0.9)) or to oppose vaccination (OR(95%CI): 0.3(0.1-0.9)). The majority (>60%) indicated to be well informed about the risks of influenza and the efficacy of the vaccine. The main reason for vaccination in those who previously refused it was resident protection. The manual was found useful by the organizers of the campaigns. We conclude that the use of an intervention manual may support vaccination uptake and decrease perceived barriers towards influenza vaccination in countries without mandatory vaccination in HCWs.

Keywords: Healthcare workers; influenza; vaccination; intervention manual; campaign; long-term care.

Introduction

Institutionalized elderly often do not develop protective antibody titers after vaccination due to increased age, chronic disease or malnutrition [1,2]. Hence, seasonal influenza remains an annual threat as well as complications such as pneumonia, exacerbation of underlying cardiopulmonary disease, hospitalization or even death [3]. During nosocomial outbreaks, healthcare workers (HCWs) have an increased risk of acquiring influenza since they care for the infected residents [4]. Since influenza can be transmitted even before the onset of symptoms, HCWs can unintentionally spread influenza. HCW vaccination is the most effective measure to prevent this. It may decrease morbidity and mortality in residents and can prevent HCW absenteeism [5–8]. Despite these advantages and the recommendations made by public health authorities, vaccination coverage in HCWs remains generally low. It is about 65% in the United States and varies from 14% to 46% across Europe [9,10]. Different strategies to improve the vaccination coverage have been explored, including extensive communication, education, free-of-charge vaccination, easy access to the vaccine and even mandatory vaccination [11–13]. The latter has proven to be very effective and often leads to vaccination coverage rates of more than 90% [13]. However, mandatory vaccination is not generally accepted as there are many ethical concerns. The duty not to harm or infect residents of LTCFs conflicts with the freedom to decide whether or not to have the vaccine. As long as this debate remains ongoing, well-prepared campaigns that include multiple interventions are the best available strategy to increase vaccination coverage [11]. The World Health Organization (WHO) Regional office for Europe recommends an evidence based approach to tailor seasonal influenza immunization programs to a specific setting [14]. In this context, we developed a ready-to-use instruction manual to facilitate implementation of interventions that are known to increase vaccination coverage and that target barriers towards vaccinations in healthcare institutions [15]. We evaluated the usefulness of this manual and its impact on vaccination uptake, attitudes towards influenza vaccination and reasons for vaccine acceptance in 11 long-term care facilities (LTCFs) during the vaccination campaign preceding the 2017/2018 influenza season.

Methods

Development of the instruction manual for the organization of vaccination campaigns

The manual contains a stepwise approach with 24 possible interventions ranging from preparatory work to campaign evaluation (table 1). Potential interventions were based on best practices in our previous study added with interventions from published studies and, campaign methodologies used in other countries and guidelines from health authorities [11–14,16–20]. Studies were identified in Pubmed with the following keywords: "vaccine", "vaccination", "immunization", "flu", "influenza", "healthcare workers", "transmission", "motivation" and "intervention". Additional publications were found in references of those articles by the snowball effect. Subsequently, interventions to be included were chosen specifically to target determinants of vaccination uptake in Flemish LTCFs and to fit the Easy-Attractive-Social-Timely (EAST) model for behavioral change. [15,21]. The feasibility of implementing these interventions in Flemish healthcare institutions was discussed during round table sessions with flu campaign coordinators from LTCFs. Lastly, feedback from different stakeholders (health psychologist and campaign coordinators of LTCFs) was asked before finalizing the manual. The manual is available in Dutch on http://www.laatjevaccineren.be/hou-griep-uit-je-team.

Study population and design

All participating LTCFs of a previous study on determinants of vaccination were invited to participate in a follow-up study and 11 agreed [15]. All long-term care facilities provide permanent residence and on site personal assistance with daily activities, nursing and other medical care, for persons above 65 years of age who can no longer live at home. The characteristics of the LTCF and the institution wide vaccination coverage data for the years 2014 to 2017 were obtained directly from the LTCFs. The implementation of vaccination promoting measures by the LTCF was assessed with a structured interview with the local campaign organizer before and after the intervention. One pre-intervention and one post-intervention interview was organized per participating LTCF. Pre-intervention interviews were scheduled in June 2017, before implementation of the manual, and post-intervention interviews in January 2018, after completion of the vaccination campaign. Attitudes towards vaccination and reasons for vaccine acceptance were attained with quantitative surveys in HCWs before and after the campaign. All HCWs from the participating LTCFs were asked to complete the pre-intervention survey between 31 August 2017 and 29 September 2017 and the post-intervention survey between 5 January 2018 and 2 February 2018. The surveys were anonymized using an allocation number and returned in sealed envelopes to ensure confidentiality of responses. Consent for use of the responses for scientific purposes was given on the first page of the survey, which also contained information about the design and aim of the study. Since the survey of adult healthcare workers was anonymous for the investigators, the interview with the institutional influenza campaign organizers only covered the practical organization of influenza campaigns (and no personal views on vaccination), and the intervention comprised regular practice, this study was exempt from a review by the ethical board of the university hospital.

Table 1: Implemented interventions from the instruction manual

Category	Goal	Interventions	LTCF that used intervention in 2016 (n)	LTCF that used intervention in 2017 (n)	LTCFs that used intervention in 2016 and 2017 (n)
Campaign management	Counter organizational barriers	1. **Multidisciplinary organizational team** consisting of at least three persons	5	10	5
		2. **Evaluation of previous campaign** with evaluation document attached in manual.	0	4	0
		3. Defining a campaign **goal** (e.g 10% increase in vaccination coverage)	5	7	5
		4. Use of a **step-wise action plan** for the preparation of a vaccination campaign (attached in manual)	0	5	0
		5. **Evaluation of campaign** with evaluation document attached in manual	0	1	0
		6. **Electronic registration** of vaccination	9	9	8
		7. Providing **Feedback** about campaign results to HCWs*	1	10	1
Education, communication and promotion	Encourage vaccination, raise awareness about importance of vaccination for resident and own protection, increase knowledge about vaccination and combat myths and disbeliefs	8. **Vaccination campaign kick-off event** (information session for staff at start of campaign, whether or not with vaccination moment involved)	1	7	1
		9. **Personal incentives**	1	3	1
		10. **Group incentives** (e.g. incentive for specific wards or whole LTCF† if predetermined goal is attained)	6	6	5
		11. Awarding wards or whole LTCFs with a **certificate** if a certain goal is reached	0	7	0
		12. Use of **campaign image** (vaccinated healthcare workers forming a protective circle around a vulnerable resident)	0	10	0
		13. Use of a **personalized campaign** with the own personnel as the main figures	0	10	0
		14. **Communication messages** with sufficient information (more than only vaccination data)	6	9	6
		15. Use of **general communication channels** (posters/flyers/screens)	11	11	11
		16. Use of **personal communication channels** (mail/letter)	5	7	5
		17. **Education session for supervisors**	1	4	1
		18. **Education session for healthcare workers**	6	8	5
		19. Use of **educational material** attached in manual (fact sheets and myth busting sheets)	0	8	0
		20. **Active involvement of supervisors or role models** in the campaign (e.g. one to one vaccination encouragement by supervisor or visible vaccination of a supervisor)	9	11	9
Easy-access	Allow easy access vaccination and counter organizational barriers.	21. Vaccination **without prior enrolment**	3	5	3
		22. **Vaccination at easy-accessible and commonly used locations**	4	9	4
		23. **Multiple vaccination moments**	4	10	4
		24. **Peer vaccination**	7	10	7

**HCW: healthcare worker; †LTCF: long-term care facility.*

Training and interviews with vaccination campaign coordinators

The structured pre-intervention interview in 2017 contained questions on the measures used during the vaccination campaign preceding the 2016/2017 influenza season. Afterwards, coordinators were trained on the use and content of the manual and the potential vaccination promoting actions listed in table 1. During the structured post-intervention interview, we evaluated which interventions from the manual were actually implemented. In addition, the coordinators were asked to rate their satisfaction with the manual on 10-point Likert-scale and also to provide feedback on the usability. The pre-intervention interview took approximately 20 minutes while the evaluation interview lasted about 40 minutes since all interventions were assessed one by one.

Healthcare worker survey

The surveys were based on previously used questionnaires to ensure comparability [22–24]. The pre-intervention survey consisted of three parts: (i) demographics; (ii) reasons for (non)vaccination (iii) attitudes towards influenza vaccination. Additionally, the post-intervention survey also contained questions on the visibility and utility of the vaccination campaign.

Statistical analysis

The pre- and post-intervention surveys were entered in an MS Access database. The distributions of characteristics pre and post intervention survey respondents were compared to the total sample and to census data using a chi-squared goodness of fit test. Census data concerned population data of adult healthcare workers employed in Flemish long-term care facilities of the same type (i.e elderly care) as those in our study. The change in vaccination uptake after the 2017-campaign compared to the 3 preceding years was tested with repeated measures ANOVA with Bonferroni correction for multiple testing. Linear regression was used to estimate the increase in vaccination coverage in relation to the number of newly implemented interventions. Attitudes regarding vaccination were recorded on a 5-point Likert scale which was dichotomized by combining (i) “strongly agree” and “agree” as a positive response and (ii) “do not agree/do not disagree”, “disagree” and “strongly disagree” as a negative response. Generalized linear mixed-effects models (mixed effects logistic regression) were used to determine the effect of the intervention on attitudes. The models included the subject nested within the relevant LTCF as a random factor and have the advantage over traditional pre-post intervention models like an(co)va that they use information from all participants, irrespective whether they completed only one or both surveys. Since there was a substantial non-response to either survey, characteristics of the respondents of the first and second survey were compared with the total sample using a chi-squared goodness of fit test. In addition, Pearson Chi^2 tests were used to compare the characteristics between the respondents of the first only, second only or both surveys. A test probability of 5% was considered statistically significant. All data were analyzed with R. version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, 2013).

Results

Characteristics of participating long-term care facilities and healthcare workers

The eleven participating LTCFs employed a median number of 120 HCWs (range: 40-170) and had a median number of 121 beds (range: 65-161). All LTCF participated in both surveys. Approximately 1250 HCWs received both surveys. In total 829 participants (response rate 66.1%) completed at least one of both surveys. The pre-intervention survey was completed by 645 HCWs (response rate: 51.4%), the post-intervention survey by 524 HCWs (response rate: 41.8%) and 340 HCWs completed both. Data from one pre-intervention participant were excluded from the analysis because the vaccination status was unclear which rendered most of the questionnaire unusable. Characteristics of survey respondents are listed in table 2. No statistically significant difference was observed when respondents to the first and second survey were compared to the total sample (Pearson Chi² goodness of fit test, all >0.05) (table 2). When the characteristics were compared by response group (only the first, only the second or both surveys), females were more likely to answer both surveys (42.1% versus 36.7% in males, p = 0.02). All other characteristics did not significantly differ by response group (data not shown). A large majority were women and the median age was 44 years as well pre and post intervention. Participant characteristics were comparable to census data on LTCF staff, with the exception of a slightly larger proportion of participants >50 years of age (36% versus 25%; p<0.001) and nurses (23% versus 19%;p<0.01) [25].

Table 2: Personal characteristics, occupation and vaccination status of survey participants

	***All HCWs†* (n=828)**	***Pre-intervention* (N =644)**	***Post-intervention* (N=524)**
	%	%	%
Personal data ‡			
Female	88.0	89.7	86.9
Median age, years (range) §	-	44 (18-65)	44 (20-62)
Having chronic illness	7.9	7.1	7.0
Having children at home	46.1	44.7	46.1
Having elderly at home	6.5	5.6	7.2
Having chronically ill person at home	6.4	5.6	6.9
Occupation			
Function			
Nurse	23.4	23.6	24.8
Nursing Aides	34.9	33.7	33.6
Other HCWs with resident contact ¥	11.6	11.6	11.8
Other HCWs without resident contact ‖	27.5	28.6	28.2
Other HCWs, unknown function	2.5	2.5	1.5
Daily contact with residents	92.1	91.7	91.3
Influenza vaccination status		**%**	**%**
Vaccinated during the previous campaign (2016 or 2017, respectively)	-	58.7	70.2
Never been vaccinated	14.6	18.5**	13.9
Annually vaccinated	51.6	51.2	55.9 *

† HCW: Healthcare worker
‡ For each item, between 1.2% and 1.7% of the respondents did not complete the particular question, except for item 'having a chronically ill person at home' and 'having a child at home, it was 15.6% and 16.1%, respectively.
§ Median age for all HCWs group was not described as age changed between the two time points (pre or post intervention).
¥ Pharmacists, audiologists, physiotherapists, paramedics, psychologists, animation
‖ Medical technical staff, administrative, facilities and logistics
Chi² goodness of fit test (pre or post intervention versus all respondents): *p<0.05 **p<0.01

Implementation and usability of the manual: results from structured interviews

The number of LTCFs that implemented a particular intervention from the manual in 2016 (before) and in 2017 (after the intervention) is shown in table 1. The local campaign coordinators rated a median score of 8 (range 5-9.5) on a 10-point Likert-scale for satisfaction with the use of the manual. One LTCF preferred not to rate the manual as they had only implemented two new interventions. The LTCF that scored 5 had a pre-intervention vaccination coverage of 72% and considered the manual more useful for LTCFs with a low pre-intervention vaccine uptake. The other LTCFs were very positive about the use of the manual and stated that it was practical, encouraging and inspiring.

Vaccination uptake reported by the LTCF

After implementation of the manual, the mean vaccination coverage reported by the 11 LTCFs significantly increased from 54% (range: 35-72%) in 2016 to 68% (range: 45-81%) in 2017 ($p<0.05$, repeated measures ANOVA with Bonferroni correction). Conversely, no significant increase with the preceding year could be found in 2016 or 2015 (figure 1A). A 10-30% increase is found in the 9 LTCFs that implemented 7 or more new interventions described in the manual (figure 1B). In general, the vaccination coverage is estimated to increase 1.6% (95% confidence interval: 0.2-2.9; $p<0.05$) per newly implemented intervention (figure 1C).

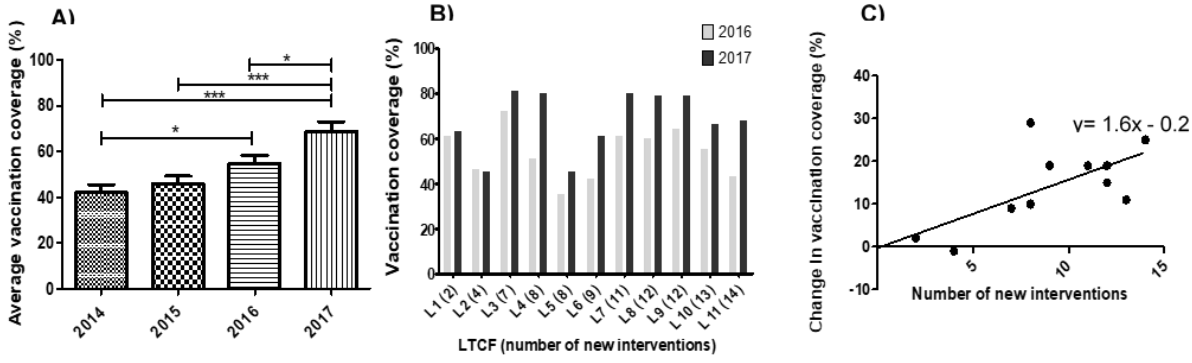

Figure 1 Influence of campaign organized with instruction manual on vaccination coverage. A) Mean vaccination coverage in participating LTCFs three years before and the year after the implementation of the manual. Change in coverage rates are determined with repeated measures ANOVA. One LTCF was excluded for this analysis due to missing data. This LTCF only opened in 2016. B) Vaccination coverage before and after implementation of the instruction manual. Participating LTCFs are ordered from L1 to L11 according to the number of implemented interventions. C) Change in vaccination coverage in relation to number of newly implemented interventions. Each data point represents one participating long-term care facility (LTCF). *p<0.05; **p<0.01; ***p<0.001

Vaccination uptake before and after implementation of the manual as reported by the HCWs

The vaccination uptake reported by the survey respondents was 58.7% in the 2016 and 70.2% in 2017. Of the 340 participants that completed both the pre- and post-intervention questionnaire, 140 were not vaccinated in 2016 but 52 (37.1%) of them were subsequently vaccinated in 2017. Among the in 2016 non-vaccinated HCWs, 64 (45.7%) had never been vaccinated against influenza and 18 (28.1%) of them received the vaccine for the first time in 2017.

Attitudes and beliefs regarding influenza vaccination before and after implementation of the instruction manual

Table 3 represents attitudes and beliefs in HCWs before and after the vaccination campaign. The first column shows the prevalence of agreement with a particular statement concerning vaccination before the campaign and the second column the prevalence after the campaign in all respondents who completed these surveys. The third column shows the odds ratio of agreeing with certain statement after the campaign compared to before the campaign as determined with the mixed model logistic regression. Positive changes in attitudes and beliefs were mostly observed in the domain of perceived barriers to influenza vaccination. HCWs were for example less likely to expect side effects after influenza vaccination (p=0.03), to underestimate the danger of influenza (p=0.001), or to oppose vaccination in general (p=0.04). On the other hand, HCWs were less likely to consider themselves as a risk group for influenza (p=0.01). Although the majority of HCWs (>90%) finds it important not to infect residents, they were less likely to agree with this after the campaign (p=0.02). The statistical model used in table 3 not only considers the pre- and post-intervention responses but also the correlated responses of respondents who completed both surveys, and those who belong to the same LCTF. The model may therefore indicate a change that goes in the opposite direction to what the prevalence data suggest. However, for the item “I oppose vaccination in general” the trend towards less agreement was confirmed when directly comparing responses in subjects who answered both surveys (data not shown). Also, the prevalence of those that agreed with the items ‘I am especially against vaccination in HCWs’ and ‘I think my employer only offer influenza vaccination to reduce costs’ is very low and does not differ much pre and post intervention. Therefore, the significance level of the odds ratios of these items should be interpreted with caution.

Table 3: Attitudes and beliefs regarding influenza vaccination pre and post intervention (generalized linear mixed effect models)

	Survey respondents (N = 828)		
	Pre-intervention	*Post-intervention*	OR[†] (95 % CI) [‡]
N:	644	524	
Attitude	*(%)*	*(%)*	
I find it important that:			
• HCWs[§] do not infect residents.	*91.6*	*90.2*	*0.2 (0.6-0.8)**
• All HCWs are vaccinated against influenza to ensure continuity of care.	*56.0*	*57.3*	*1.0 (0.7-1.4)*
• HCWs have the freedom to decide whether or not to have the influenza vaccine	*79.9*	*78.2*	*0.8 (0.4-1.7)*
I consider influenza vaccination important as it is my duty not to harm residents	*54.8*	*55.8*	*1.1 (0.8-1.4)*
I think influenza vaccination should be mandatory for HCWs	*20.6*	*22.4*	*1.8 (0.8-3.8)*
Advantages of influenza vaccination	*(%)*	*(%)*	
Vaccination against influenza gives me confidence that:			
• I will not get influenza	*58.4*	*50.4*	*1.1 (0.8-1.5)*
• I will not infect residents	*57.3*	*59.8*	*1.2 (0.9-1.6)*
• I will not infect my family	*58.1*	*59.6*	*1.1 (0.8-1.5)*
I find it important that all HCWs are vaccinated in order to avoid increased workload	*52.5*	*57.5*	*1.3 (1.0-1.8)°*
Perceived barriers against vaccination	*(%)*	*(%)*	
I think influenza is not dangerous to me	*21.2*	*15.0*	*0.2 (0.1-0.5)****
Vaccination weakens my immune system	*25.3*	*23.3*	*0.7 (0.4-1.4)*
I can get the flu from the vaccine	*29.8*	*25.7*	*0.8 (0.6-1.1)*
I oppose vaccination in general	*10.8*	*11.3*	*0.3 (0.1-0.9)**
I am especially against vaccination in HCWs	*3.9*	*3.4*	*0.4 (0.1-2.0)*
I think my employer only offers influenza vaccination to reduce costs	6.1	5.3	1.8 (0.5-6.0) [¥]
If I take up influenza vaccination once, I have to do this every year	26.0	19.1	0.2 (0.1-0.3)***
I would expect to have side effects if I got vaccinated against influenza	21.2	16.7	0.4 (0.2-0.9)*
I think HCW vaccination is of little use as residents frequently have visitors.	17.0	14.0	0.3 (0.1-0.7)**
I think that LTCFs only implement influenza vaccination to prevent HWCs from being sick.	28.2	20.9	0.6 (0.5-0.9)**
Perceived susceptibility to influenza	*(%)*	*(%)*	
I think I have a high chance of getting influenza	*37.2*	*37.2*	*1.0 (0.7 -1.3)*
I think influenza is very dangerous for my residents	*88.6*	*88.3*	*0.7 (0.4-2.0)*
I think I have a high chance to infect residents	*73.0*	*74.4*	*1.0 (0.5-2.0)*
I think HCWS have an increased risk of getting ill during an influenza epidemic	*79.0*	*73.1*	*0.4 (0.2-0.8)**
I think that when I am vaccinated against the flu, I have less chance to acquire influenza compared to vaccinated residents	45.7	46.2	1.0 (0.8-1.3)

[†] Odds Ratio (OR) from a generalized linear mixed effects model with subject ID nested within LTCF as a random factor, except for [¥] because the model with nested effects did not converge: * p < 0.05, ** p < 0.01, *** p < 0.001, °p < 0,1 (trend)
[‡]CI: confidence interval; [§]HCW: Healthcare worker.
For each item, between 1.7% and 5.4% of the pre-intervention respondents and between 2.7 and 5.3 of the post-intervention respondents did not complete the particular question.

Reasons for vaccination uptake after previous refusal or missing vaccination

The main motivators for vaccination uptake in 2017 in HCWs who were not vaccinated in 2016 were protection of residents (69.2%), family members (59.6%) or themselves (53.8%). The main reasons for non-vaccination in the preceding year were concerns about efficacy (26.9%) usefulness (13.5%) and necessity of influenza vaccination (23.1%), forgot to get the vaccine (17.3%) or fear of side effects (13.5%).

Similarly, the main reasons for vaccination in those who received their first vaccine in 2017 were protection of residents (72.2%), family members (50%) and themselves (38.9%) as well as recommendation by a supervisor (44.4%). The main reasons for previous refusal were doubts about efficacy (33.3%) and the necessity of the vaccine (16.7%), never having had influenza (27.8%) and being afraid of needles (16.7%).

Perception of the campaign by the survey respondents

Of the HCW respondents, 68.8% found the campaign informative and most HCWs indicated to be well informed during the campaign about the risks of influenza (67.2%), transmitting influenza to residents (70.4%) and the safety and efficacy of the vaccine (63.3%). Moreover, half of the respondents (51.1%) stated that the campaign had made them think about the usefulness of influenza vaccination and 17.7% stated that the campaign had influenced their decision whether or not to get vaccinated.

Discussion

Higher vaccination uptake rates and decreased perceived barriers towards influenza vaccination were observed in LTCFS that used an instruction manual, which was aimed at facilitating the implementation of interventions to increase vaccination coverage. The vaccination uptake was significantly higher in 2017 compared to 2016 and the two preceding years, while in contrast no significant annual increase was observed between 2014 and 2015 and between 2015 and 2016. This assures us that the findings are not due to an ongoing trend of increasing coverage, but can be attributed to the use of the manual. Particularly, the coverage increased with 10-30% in LTCFs that implemented at least seven out of 24 possible interventions. Three LTCFs reached or exceeded the target of 80% set by the Flemish government and two approached it with a vaccination coverage of 79%. In line with the literature, we found that the degree of increase in vaccination uptake was proportional with the number of implemented measures [11,26]. Reviews on intervention programs showed that a combination of education, promotion and improved access is more effective than single intervention programs [11–13]. Therefore, we strongly focused on education, role models, personalized communication, easy access and resident- and self-protection. The added value of the manual is not only suggested by the increased coverage, but even more so by the finding that nearly 40% of those who were not vaccinated in 2016 and nearly 30% of those who had never been vaccinated, received the vaccine in 2017.

Furthermore, after implementing the manual, we observed a significant decrease in perceived barriers towards vaccination. HCWs were less likely to expect side effects, to oppose vaccination, to believe that influenza vaccination is used to decrease sick leave or to believe that vaccination is useless due to the many visitors the residents have. Strangely, we also found that HCWs were less likely to give importance

to not infecting residents. However, a large majority (>90%) still agreed with this statement. Llupía *et a*l [27], found that HCWs perceived influenza as a more severe disease after a multi-intervention campaign. Similarly, we found that HCWs were more likely to think that influenza might be dangerous. Contrariwise, they were less likely to believe they have a higher risk of falling ill from influenza. It is possible that HCWs realize that influenza can be dangerous but that they gained trust in the vaccine efficacy and hence do not expect to become infected. Trust might be gained through the educational part of the campaign. This is supported by the fact that most LTCFs implemented the educational material from the manual and that HCWs indicated to be well informed about the risk of influenza and the safety and efficacy of the vaccine. Additionally, we found that previously non-vaccinated HCWs who were sceptic towards the vaccine, can change their mind towards vaccination. Interestingly, in those HCWs, resident protection was the mind-changing driver. This seems logical as 70% of HCWs indicated to be well informed about the risk of transmission to residents during influenza epidemics, but it is also surprising given that self-protection has consistently been reported as the most important driver for vaccine acceptance in HCWs [11]. Given this, we believe that focusing on the combination of HCW and resident vaccination is the best available means of protecting residents.

A strength of the manual is that the interventions were specifically tailored to healthcare institutions. This is important as peer opinions, cultural and institutional factors influence knowledge and behavior in specific settings [11]. Also, the organizers of the campaigns found the manual practical in use. This assures us that other LTCFs will equally be able to use it in a convenient way. The study also had a large number of study respondents, which reassures us that the data are robust. Moreover, the LTCF which did only use a limited number of interventions did not see an increase in vaccination coverage.

Nevertheless, there were also some limitations involved in this study. Firstly, we did not have of a control group of LTCFs with similar pre-intervention vaccination uptake rates. We can therefore not rule out the possible effect of outside factors such as exposure to information in the media. However, both 2016-2017 and 2017-2018 were moderate influenza seasons with limited media attention. Another limitation is that there was heterogeneity between the campaigns. Since there were 24 possible interventions and only 11 participating LTCFs, the study was not sufficiently powered to analyze the impact of the individual interventions. Yet we believe that combining multiple interventions is effective to improve vaccination uptake [11–13]. Thirdly, the response rate was limited and a large number of participants completed only one survey. Nevertheless, the response rate of 51.4% for the pre-intervention survey and 41.8% for the post-intervention survey is higher than the 29% observed in LTCF in a previous study and in line with what is seen in other studies [15]·[22–24]. We also believe that the data are robust as the majority of characteristics of those who completed both surveys were not statistically different from those who completed only the first or second survey. Furthermore, the manual was only evaluated during one campaign. Therefore, we cannot ascertain that the findings are sustainable. However, previous influenza vaccination has shown to be a good predictor of future vaccination uptake [11]. Additionally, evidence exists that maintained efforts go along with high and sustained vaccination rates [11]. For example, a recent study

on a multi-intervention program found an increase from 70 to 90% in two years' time and a sustained coverage of 90% in the subsequent year [28]. Although we did not assess long-term effects, flu coordinators showed the intention to continue using the manual to organize their campaigns because they observed a significant increase in vaccination coverage. Finally, there were small differences between the demographic profile of the survey participants and census data. This is not likely to have a large impact on the generalizability of our results because the census data relate to full-time equivalent HCWs whereas we counted all healthcare workers irrespective of working time.

In summary, we conclude that the intervention manual supports vaccination uptake and decreases perceived barriers towards influenza vaccination. This is especially true when LTCFs are motivated and willing to invest time and financial resources. These results should encourage competent authorities to create a similar ready-to-use manual to facilitate the implementation of interventions that are known to increase vaccination coverage in healthcare institutions. Even if vaccination is mandatory, this manual might still be useful to increase awareness and acceptance by the HCW, who may otherwise take exception to such a top-down policy.

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CHAPTER 9: Concluding discussion and future perspectives

Concluding discussion

During the past decade several outbreaks with vaccine-preventable diseases such as measles, mumps and pertussis have occurred. Annual influenza epidemics also pose a recurring threat to individuals at risk and healthcare institutions. Moreover, many patients are still at risk of developing cancers induced by acquired infectious pathogens, such as HPV or HBV. Some individuals are at increased risk of complications following infection with a microbe due to age, underlying disease or immunosuppressive treatment. Except for the availability of clean water and sanitation, the impact of routine practice of vaccination on lowering morbidity and mortality cannot be exaggerated. However, research on vaccines in at-risk groups, who have the greatest need of protection, is limited. With this PhD project, we aimed to increase the understanding of direct and indirect protection induced by vaccines in at-risk patients.

A first way in which vaccines protect vulnerable at-risk patients is by direct protection. This is the protection provided to an individual by an immune response following vaccination. In this context, we firstly addressed immunogenicity, which is the ability of a vaccine to induce an immune reaction. We assessed immunogenicity and safety of the 9vHPV vaccine in 100 HIV and 171 solid organ transplantation (SOT) patients by measuring antibodies as a measure for the humoral immune response. This was the first study that evaluated the 9vHPV vaccine in these populations. The 3-dose regimen with 9vHPV vaccine was well tolerated in both patient groups. We found that all HIV patients seroconverted for all HPV types, but seroconversion ranged from 46% for HPV45 to 72% for HPV58 in SOT patients. Among HIV patients, significantly higher titers were reached in patients with an African origin compared to Caucasians for all HPV type except 6 and 11. There was no clear effect of the CD4 count. Among SOT patients, seroconversion was lower for all studied HPV types when the patient received mycophenolate mofetil or tacrolimus, albeit only significant for mycophenolate mofetil. For HIV patients, the immunogenicity outcomes were slightly better to what was found with the qHPV vaccine in HIV-infected women between 13 and 45 years, which is likely related to the fact that all our patients had a CD4 count of at least 200 cells/µl prior to the start of vaccination [1]. Compared to healthy young adults, the GMTs are in the same range for HPV types 6 and 11 but slightly lower for HPV types 16/18/31/33/45/52/58 [2–5]. This could be due to the older age of the patients in our study. Regarding SOT patients, the immunogenicity outcomes are lower than what was seen in healthy adults, but in line with the findings of the qHPV vaccine in SOT patients [2–6]. Given the high burden of HPV disease in HIV and SOT patients, the 9vHPV vaccine is beneficial as it covers a broad range of HPV types. Importantly, HPV vaccination has no therapeutic effect on HPV infections at the time of vaccination, but can still prevent infection of other HPV-types. This is valuable as for each individual HPV type, at least 65% and up to 95% of the HIV patients and more than 90% of SOT patients were seronegative. Since no complete protection against HPV disease can be guaranteed after vaccination in SOT patients, we advocate vaccination before transplantation in combination with post-transplant screening at regular time intervals and reimbursement of these expensive

vaccines. However, if vaccination was not possible or done before transplantation, the best way to protect these patients is to combine post transplantation vaccination with frequent regular screening.

In order to have a broader idea about direct protection in at-risk groups, we performed cross-sectional studies in which we assessed vaccination status and seroprevalence of antibodies of standardly recommended vaccines. First, we evaluated vaccination coverage of influenza, pneumococcal, hepatitis B and diphtheria, tetanus and pertussis vaccines and determinants of vaccination in adults with chronic diseases. We included patients with diabetes mellitus type 1 (n= 173) and type 2 (n=177), chronic kidney disease (CKD) (n=138), heart failure (n=200), chronic obstructive pulmonary disease (COPD) (n=187), HIV (n=201) or a history of a SOT (n=255) in a monocentric study. We found suboptimal vaccination coverage for all studied vaccines, ranging from 10% for pertussis to 44% for influenza. Since we only considered documented vaccination, there may be an underestimation of the true coverage rates. The central vaccine register (Vaccinnet) in Flanders (Belgium) could resolve this issue, but it is not yet being used systemically for vaccines that are not available free of charge. Nevertheless, our results are in line with the influenza vaccination uptake rates reported by WHO European region which were mostly below 40% for people with chronic illnesses in 14 other European countries [7]. For influenza, the vaccination coverage is far below the WHO/EU target of 75% for at-risk groups [8,9]. Similarly, for pneumococcal vaccination, other studies reported low coverage rates ranging from 7% in Italy to 50% in immunocompromised patients in the United states and 60% in high-risk groups in Catalonia (Spain) [10–13]. Older patients were more likely to be vaccinated against influenza and pneumococcal disease. However, younger patients with chronic diseases are also at increased risk of complications, and neither recommendations nor uptake of vaccination should be different. Concerning influenza vaccination, concerns about effectiveness and side effects were important reasons for lower uptake. The most prevalent reason for non-vaccination against pneumococcal disease was not being aware of the recommendation. Given this, we urge all physicians to discuss vaccination and address reasons for non-vaccination with their patients. Another potential reason for low coverage rates is that at-risk patients are closely monitored by a specialist and therefore less often consult a general practitioner, which is the preferred vaccinator in Belgium. Specialists do not always talk to patients about vaccination as this is considered the general practitioner's task [14]. A vaccination recommendation by a specialist could thus have a substantial impact on the vaccination rate. Moreover, specific education in vaccinology for medical doctors and nurses should increase specialists' awareness of the issue and encourage them to recommend vaccines. Currently, vaccine education is limited in the training of both physicians and nurses.

Thirdly, we assessed seroprevalence of antibodies against diphtheria, tetanus and pertussis in adult at-risk patients with diabetes mellitus type 1 (DM1) (n=172), DM2 (n=77), chronic kidney disease (n=130), chronic obstructive pulmonary disease (COPD) (n=170), heart failure (n=77), HIV (n=196) or a SOT (n=230). Furthermore, seroprevalence of antibodies against measles, mumps, rubella, diphtheria, tetanus and pertussis was evaluated in 222 pediatric at-risk patients, aged 2-21 years, with allergies (n=14), congenital heart disease (n=25), diabetes type 1 (n=58), cystic fibrosis (n=9), primary immunodeficiency (n=88) or a

history of a SOT (n=28). We found that, except for tetanus, a large group of adult and pediatric at-risk patients remained susceptible for the studied vaccine preventable diseases. Firstly, this might be due to the fact that they were sub-optimally vaccinated. Among adults, seroprotection against diphtheria and tetanus and pertussis seropositivity were higher ($p<0.001$) when vaccinated <10 years ago. Likewise for children with chronic diseases, we found that age-appropriate vaccination increased seroprevalence and seroprotection. We therefore advocate checking vaccination status at each encounter with a physician. Secondly, it might be that vaccines induce lower antibody titers compared to healthy persons. We have for example demonstrated low titers among SOT patients in our 9vHPV immunogenicity study. In pediatric patients, seroprevalence rates were particularly low in SOT recipients, which again advocates for increased attention to pre-transplantation vaccination.

Since at-risk patients are not always protected against vaccine-preventable diseases it is important to combine direct vaccination with indirect protection. Resurging cases of measles, mumps and pertussis in the past decade indicate that herd protection is inadequate, and that a relatively large number of vulnerable patients with chronic disease may be at risk not only of catching the disease, but especially develop complications such as meningitis and encephalitis. Due to the lack of recent data on seroprevalence, it is not clear if these patients can benefit from community immunity. A particular form of indirect protection is cocoon-vaccination of close contacts of at-risk patients. This includes household members but also healthcare workers who care for at-risk patients in hospitals or long-term care facilities. In this context, we assessed vaccination coverage and determinants of vaccination in more than 5000 HCWs from 13 hospitals and 14 long-term care facilities. We found a vaccination coverage of about 40% in the hospitals and about 45% in the LTCFs. This is in accordance with the finding of 14% to 46% vaccination coverage in other European countries [15]. Our findings on demographic factors associated with vaccination were largely in agreement with those from previous studies, such as increasing age, male gender, chronical illness, increasing number of years of working in the healthcare sector, and higher education [16,17]. Interestingly, up to 90% of HCWs found it important not to infect their patients. However, only 20% of non-vaccinated HCWs considered influenza vaccination a duty not to harm their patients. Up to 40% of unvaccinated staff believed they could get influenza after vaccination and that vaccination weakens their immune system. Also, only about 20% of unvaccinated staff thought to have a high chance of getting influenza. Reasons for unvaccinated staff to get vaccinated in the future were self-protection and protection of family members. Factors that positively influenced vaccination coverage were encouragement by supervisors (OR, hospitals: 7.1, $p < 0.001$; nursing homes: 7.5, $p < 0.001$) and well-organized vaccination campaigns with on-site vaccination. Factors that negatively affected vaccination coverage were misconceptions about influenza and its vaccine (OR, range 0.1–0.7, $p < 0.001$ for most misconceptions) and underestimation of the risk of contracting influenza by patients or HCWs (OR of perceived susceptibility, range 2.1–5.1, $p < 0.001$ for most factors). The World Health Organization (WHO) Regional office for Europe recommends an evidence based approach to tailor seasonal influenza immunization programs to a specific setting [18]. In this context, we developed a ready-to-use instruction manual to facilitate implementation of interventions that are known

to increase vaccination coverage and that target barriers towards vaccination in healthcare institutions [19]. The manual contains a stepwise approach with 24 possible interventions ranging from preparatory work to campaign evaluation. It includes easy-access vaccination, role model involvement, personalized promotional material, education and extensive communication. The manual is available at the website of the Flemish agency for Health and Care (http://www.laatjevaccineren.be/hou-griep-uit-je-team). We evaluated the usefulness of this manual and its impact on vaccination uptake, attitudes towards influenza vaccination and reasons for vaccine acceptance in 11 LTCFs during the vaccination campaign preceding the 2017/2018 influenza season. Vaccination coverage before and after the campaign was recorded by the LTCFs and the usefulness of the manual was assessed by interviewing the organizers of the local campaigns. Attitudes towards vaccination and reasons for vaccination were evaluated with a quantitative survey in HCWs before and after the campaign. We found higher vaccination uptake rates and decreased perceived barriers towards influenza vaccination in LTCFs that used the instruction manual. In particular, the mean vaccination coverage reported by the LTCFs was 54% (range: 35-72%) in 2016 and 68% (range: 45-81%) in 2017. The coverage increased with 10-30% in LTCFs that implemented at least seven out of 24 possible interventions. Three LTCFs reached or exceeded the target of 80% set by the Flemish government and two approached it with a vaccination coverage of 79%. Since there were 24 possible interventions and only 11 participating LTCFs, the study was not sufficiently powered to analyze the impact of the individual interventions. Yet we believe that combining multiple interventions is effective to improve vaccination uptake [20–22]. We also found that HCWs were less likely to expect side effects, to oppose to vaccination, to believe that influenza vaccination is used to decrease sick leave or to believe that vaccination is useless due to the many visitors the residents have. The majority (>60%) indicated to be well informed about the risks of influenza and the efficacy of the vaccine during the campaign. The manual was found useful by the organizers of the campaigns. Based on these results, we conclude that the intervention manual supports vaccination uptake and decreases perceived barriers towards influenza vaccination. This is especially true when LTCFs are motivated and willing to invest time and financial resources. This approach might also be better accepted by HCWs as this is a bottom-up approach. The alternative is mandatory vaccination (as a top-down policy) which would be less accepted, also by HCWs in favor of seasonal influenza vaccination as was shown in our large survey in Flemish HCWs. These results should encourage competent authorities in other countries to create a similar ready-to-use manual to facilitate the implementation of interventions that are known to increase vaccination coverage in healthcare institutions.

A general limitation for most studies included in this thesis is the lack of control groups, which makes it hard to draw straightforward conclusions. Even though for some studies (e.g. chapter three), similar research has been performed in healthy populations, the comparison is hampered by a different demographic profile of the study sample (i.e. proportion male/female; age distribution). For chapter four, the comparison is additionally impeded by variation in data sampling methodologies (i.e. documented versus self-reported vaccination). With regards to the seroprevalence studies, there are only few studies in healthy population

to compare with. Moreover, comparison is hindered by differences in vaccination policy, as well as variations in the disease epidemiology, choice of serological tests and seroprevalence cut-off values.

Future perspectives

Direct protection

Research on vaccination in at-risk groups is scarce. It is, however, necessary since these groups have a high need of the protective benefits of vaccination given the increased risk of complications following infection. Increased insights in vaccine-induced direct protection of at-risk patients will enable tailoring of the vaccination programs and schedules to the specific needs of at-risk patients.

First, efforts should be made to improve direct protection to at-risk groups by increasing vaccination coverage. The guide to Tailoring Immunization Programs (TIP) from the World Health Organization could be used to tailor interventions to lower local barriers to vaccination [23]. This is a stepwise theoretical framework based on behavioral sciences, social marketing and qualitative and quantitative research. It is not only focused on the supply of vaccines, but also on the broad range of personal (behavior, attitude, beliefs), cultural, organizational, legislative, structural factors that influence vaccination uptake. An asset of this approach is that it starts with a thorough analysis of the current local situation. In this process countries are encouraged to identify groups with low vaccination uptake and to assess barriers and motivators for vaccination in these groups. Subsequently, it recommends to plan a program of interventions based the local barriers and motivators to vaccination. Thereafter, it is encouraged to evaluate the interventions and adjust them if necessary. In this context, a broader vaccination coverage and seroprevalence study in different regions in Belgium would be important to assess whether our findings could be extrapolated to persons with chronic disease from the community. This would also broaden the insights regarding which patient groups are less well vaccinated and less well protected against vaccine-preventable diseases and would enable prioritizing groups for interventions. Based on the results of barriers and motivators for vaccination already obtained in this PhD project, we recommend implementing well-organized multi-intervention vaccination campaigns in which improving recordkeeping of administered vaccines and vaccination recommendations to patients by healthcare professionals are key components.

Currently, registration of vaccines in Belgium is dispersed over the regions, since prevention is a regional matter. In Flanders you have Vaccinnet which started from the vaccine register from Kind en Gezin in 1999. Gradually, Vaccinnet was implemented and in 2005 school health service were obliged to register the vaccination done by the school doctors. As of 2006 GPs and pediatricians were asked to enter vaccination dates in Vaccinnet. However, software systems had to be upgraded to make exchange of vaccination data possible. Since 2014 there is an obligation to register the vaccines that are provided free of charge by the Flemish government. However, apart from some exceptions, most hospitals and preventive medical services are not yet connected to Vaccinnet. This means that a lot of vaccines administered in travel consultations and in the framework of occupational medicine are not registered in Vaccinnet. This is a missed opportunity, since this particularly useful tool is not used to its full potential. In Wallonia and Brussels E-vax was implemented only a few years ago. It is based on the same software as Vaccinnet, but currently

these are still two separate systems. Integrating these registers to one federal database would increase the availability of vaccination data of patients who live in Brussels or along the language border. Based on an integrated Belgian vaccine register it would also be useful to implement reminder applications to increase vaccination coverage in at-risk groups [24].

Other interventions to make vaccination more convenient for patients should as well be considered. They include pharmacy-based immunization or direct availability of vaccines at the physician's practice. Nowadays for all vaccines that are not provided free of charge, such as the influenza and pneumococcal vaccine, patients first have to go to their physician for a prescription, subsequently to a pharmacist to get the vaccine and then return to the physician for vaccination. On top of the logistic burden for the patient, this also increases the risk of damage to the vaccine if the cold chain is not safeguarded at the patient's home. Most evidence for pharmacy-based immunization comes from the United States where it has been implemented for more than 2 decades. Studies consistently report higher uptake of influenza and pneumococcal vaccination. However, also in countries where pharmacy-based immunization was implemented more recently, such as Canada, the United Kingdom and Portugal, increased uptake in at-risk groups is observed. In Canada, it was reported that 21% of patients who would otherwise not have been reached, were vaccinated against influenza at pharmacies. The United Kingdom reported an increased influenza vaccination uptake in elderly and other at-risk groups. Also, Portugal reported to have vaccinated 13% of patients who had never been vaccinated before [25]. Examples from these countries show that pharmacy-based immunization is successful and feasible if appropriate training, marketing, stake-holder engagement and regulatory frameworks are available [25]. Another option to improve the convenience of vaccination for the patient, is direct supply of vaccines to the physician's office. This way they can be directly provided at the first contact with the patient. Both of the above described interventions have the additional benefit of improving quality control of the vaccine as cold chain interruptions are avoided.

Furthermore, studies on vaccination coverage and seroprevalence in different countries would allow for comparison between vaccination programs, different vaccination policies, education of physicians with regard to vaccination and the way in which patients are informed about vaccination. Best practices defined in such studies could be implemented and specifically tailored to the Belgian context.

Secondly, research to improve immune response after vaccination for at-risk groups who respond less well to vaccination should be undertaken. There are several options to increase the immune response based on findings of other vaccines: 1. Increase the antigen dose (e.g. hepatitis B, influenza) 2. Add additional doses to the vaccination program (e.g. pneumococcal vaccines in premature babies, hepatitis B) 3. Use an adjuvant to improve the immune response (e.g. influenza and zoster in elderly). We found suboptimal 9vHPV vaccine immunogenicity in SOT patients but the vaccine was immunogenic in HIV patients. Hence, it should be investigated whether a supplemental dose of the 9vHPV vaccine in SOT patients would increase immunogenicity in patients who did not seroconvert. Also, long-term 9vHPV vaccine immunogenicity remains unknown in SOT and HIV patients. Another study with the qHPV vaccine found a

rapid decline in antibody titers but relatively stable seroconversion rates at 12 months in 29 adult transplant patients. An Australian study found that seroconversion was still more than 85% for all qHPV vaccine types in wide range of pediatric immunocompromised children (SOT, HSCT, juvenile idiopathic arthritis, inflammatory bowel disease) at 60 months following vaccination [26]. For persons with HIV, long-term immunity has only been shown for the bivalent and qHPV vaccine for up to 12 months after vaccination [27]. Immunogenicity should therefore be assessed with the 9vHPV vaccine over a larger period of time. This information would be relevant to see whether seroconverted patients maintain immunity or whether they need a booster dose at a later stage. Furthermore, it would be relevant to assess whether 9vHPV vaccine immunogenicity is also disrupted in other immunocompromised patients, such as patients with leukemia, hematopoietic stem cell transplantation or patients with immune mediated inflammatory diseases (e.g. rheumatoid arthritis or multiple sclerosis). Identification of patient groups in which HPV vaccines are less immunogenic, is not only important to assess the need of a booster dose but also to tailor HPV screening practice to the specific needs of a patient group. Furthermore, this area of research should be broadened to other vaccines. For example, a study showed that two standard doses of influenza vaccine is more immunogenic compared to one dose in SOT patients [28]. This would also be particularly interesting for pertussis, as these diseases have been resurging during the last decade due to waning immunity after vaccination with the acellular pertussis vaccine. Mutations that increase the pathogenicity of pertussis might as well have played a role in the resurgence of pertussis. For example, changes in the ptxP3 gene lead to an increase production of pertussis toxin, which may enhance the suppressive effects on the innate and adaptive immune system upon infection [29]. Nevertheless, implementation of booster doses has shown to be effective in the targeted populations. Since, it is supposed from seroprevalence studies that cases of pertussis are largely underestimated and that immune response to pertussis is boostable even in persons in whom the immune system is not completely functional such as elderly, it could be considered to more frequently give a pertussis booster dose to those at risk of complications. Measles has as well been resurging during the last years, but rather due to decreased vaccine uptake. In order to avoid measles cases in the population as well as in at-risk patients, all people should receive two doses.

Not only booster doses but also vaccines developed specifically for people with a decreased immune response are useful. For example, adjuvanted and high-dose influenza vaccines have already shown to elicit better immune response to vaccination in elderly and a adjuvanted hepatitis B vaccine proved to elicit a higher response in patient with chronic kidney disease [30–32].

Furthermore, large scale efficacy data of recommended vaccines in at-risk patients are scarce and would be useful to assure that vaccination is beneficial in at-risk groups. This would be especially relevant for vaccines for which no correlate of protection has been defined. However, instead of large-scale and expensive efficacy trials, connecting, anonymously, vaccine registers with disease registries would also allow the evaluation of the impact of a vaccination program on disease incidence in a given country. By combining both registries, the prevalence of a vaccine-preventable disease could be compared before and after the implementation of a vaccination program. Based on this principle Denmark was able to show the

impact of the HPV vaccination program on the incidence of high-grade squamous intraepithelial lesion in the vaccinated versus unvaccinated populations [33]. In Belgium this is, however, very complex since disease registries are federal matter and vaccine registers are part of the regional level.

Indirect protection

Vaccination of at-risk patients combined with indirect protection through herd immunity (e.g. cocoon-vaccination) is the best way to prevent vaccine-preventable disease in at-risk patients. However, at the moment it is unclear to which extent at-risk patients can be indirectly protected. There are several indications for future work. First, there is the general lack of data on protection against vaccine-preventable diseases in the general population. Large scale seroprevalence studies are needed to assess what the level of protection is in a given population and whether at-risk patients are protected by herd immunity. Based on these results targeted vaccination programs can be developed, which not only aim at increasing protection to all persons, but also specifically for at-risk groups. Secondly, there is the issue of nosocomial outbreaks of influenza. Unfortunately, the seasonal influenza vaccine is not the most efficacious available vaccine. Protection against seasonal influenza varies from year to year, but also increasing age and co-morbidities influences protection. This influences beliefs about the effectivity and usefulness of the vaccine. More in-depth studies on the immune responses and thus protection by the influenza vaccine in different groups are necessary to elucidate the very complex issues on influenza vaccination. One study in the Netherlands, for instance, found that influenza-like-illness does not decrease with influenza vaccination, even though infection with laboratory-confirmed influenza does [34]. This can give the impression that the flu vaccine is not effective and therefore is not useful. Therefore, large scale randomized-controlled trials that run over different years, during which a high vaccination coverage in HCW is reached and where laboratory-confirmed influenza as outcome measure is used are important to determine the impact of HCW vaccination on transmission of the influenza virus from HCW to patients, and to resolve the returning question on the efficacy of the seasonal influenza vaccine in preventing nosocomial influenza outbreaks.

At the moment, the best way to improve vaccination coverage in HCWs is by well-prepared multi-intervention campaigns. To this end, the manual that was created as part of this PhD project is a useful tool. As it is available online for free and available for all Flemish healthcare institutions, future research should assess how widely it has yet been implemented in LTCFs and in hospitals. In addition, it should be surveyed if healthcare institutions that have implemented interventions from the manual have seen an increase in vaccination coverage since the implementation of the manual. Information from more healthcare institutions and from control healthcare institutions, which did not use the manual, will allow to assess which interventions are most effective. In addition, satisfaction with the use of manual should be assessed on a larger scale. Based on these results, the manual could be optimized.

In the context of the current novel corona SARS CoV2 pandemic, it is possible that a second or next wave will coincide with the next influenza seasons. The simultaneous circulation of both viruses might pose substantial pressure on the healthcare system. It will be important to reach high vaccination coverage in at-

risk groups and healthcare workers. The manual we created might be a useful tool to improve vaccination coverage in healthcare workers and as such prevent an overloaded healthcare system.

General conclusion

We conclude that many at-risk patients are inadequately vaccinated and remain susceptible to vaccine-preventable diseases. For this reason, we advocate for a closer follow-up of vaccination status. To this end, it would be useful to systemically register all vaccinations in Vaccinet, regardless of whether they are provided free-of-charge. This would improve the overview of vaccination status of a patient for all treating physicians. Given the suboptimal seroconversion and the high susceptibility for other vaccine-preventable diseases in SOT recipients, we emphasize on the importance of pre-transplantation vaccination. Furthermore, to optimize protection in at-risk patient, advocate vaccinating direct contacts, especially for vaccine-preventable diseases, which are still endemic such as influenza, pertussis, measles and mumps. Vaccination of close-contacts includes vaccination of healthcare workers. We found that HCWs are sub-optimally vaccinated against influenza, but that vaccination coverage can increase after implementation of a well-organized multi-intervention campaign.

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English summary

Vaccination does not only directly protect against infectious diseases but also contributes to the protection of an entire population when a large proportion of the population (>90-95%) is vaccinated. Through herd protection vulnerable individuals (i.e. those who cannot be vaccinated and those who do not respond to vaccination) can also be protected. This PhD project includes different studies which were related to the direct and indirect protection of patients and individuals at risk.

Part I of this PhD thesis concerns direct protection after vaccination. In this context, we firstly addressed immunogenicity, which is the ability of a vaccine antigen to induce an immune reaction (**chapter 3**). Particularly, we assessed immunogenicity and safety of the 9vHPV vaccine in 100 HIV and 171 solid organ transplant (SOT) patients in a phase III investigator-initiated study. This was the first study that evaluated the 9vHPV vaccine in these populations. We found that all HIV patients developed an immune response for all HPV types, but seroconversion only ranged from 46% for HPV45 to 72% for HPV58 in SOT patients. Among HIV patients, significant higher titers were reached in patients with an African origin compared to Caucasians for all HPV type except 6 and 11. There was no clear effect of the CD4 count on immune response. Among SOT patients, seroconversion was lower for all studied HPV types when the patient received mycophenolate mofetil or tacrolimus, albeit only significant for mycophenolate mofetil. The 3-dose regimen with 9vHPV vaccine was well tolerated in both patient groups.

In a second project we assessed whether the recommended vaccination program was applied correctly in and was able provide adequate protection to risk patients. Therefore, a series of risk pediatric and adult patients were screened for their vaccination status and degree of protection measured through circulating antibodies to some vaccine preventable diseases. In **chapter 4**, we evaluated vaccination uptake of influenza, pneumococcal, hepatitis B and diphtheria, tetanus and pertussis vaccines and determinants of vaccination in adults with chronic diseases. We included patients with diabetes mellitus type 1 (n= 173) and type 2 (n=177), chronic kidney disease (CKD) (n=138), heart failure (n=200), chronic obstructive pulmonary disease (COPD) (n=187), HIV (n=201) or SOT (n=255) in a monocentric study. Overall, 29% of subjects were vaccinated against diphtheria-tetanus, 10% against pertussis, 44% against influenza, 32% against pneumococcal disease and 24% of HIV patients and 31% of CKD patients against hepatitis B. Age was positively associated with vaccination against influenza (OR:2.0, $p<0.01$) and pneumococcal disease (OR:2.6, $p<0.001$). Patients with COPD, HIV and SOT were more likely to be vaccinated against influenza (OR:2.8, $p<0.001$, OR:1.8, $p<0.05$; OR:2.0, $p<0.001$, respectively) and pneumococcal disease (OR:2.9, $p<0.001$, OR:25.0, $p<0.001$; OR:2.6, $p<0.001$, respectively) than patients with heart failure. Reason for non-vaccination were concerns about effectiveness, necessity and side effects of influenza vaccines, and not being aware of the recommendation for pneumococcal vaccination. We concluded that initiatives to monitor the vaccination status of vulnerable patients are needed, which is why we advocate systematic vaccination registration and frequent communication about vaccination.

In **chapter 5** we assessed the seroprevalence of antibodies against diphtheria, tetanus and pertussis in adult at-risk patients with diabetes mellitus type 1 (DM1) (n=172), DM2 (n=77), chronic kidney disease

(n=130), chronic obstructive pulmonary disease (COPD) (n=170), heart failure (n=77), HIV (n=196) or a SOT (n=230). Seroprotective titers were reached in 29% for diphtheria (≥0,1IU/ml), in 83% for tetanus (≥0,1IU/ml), and seropositive titers in 22% for pertussis (≥5IU/ml). Seroprotection rates were higher ($p<0.001$) when vaccinated <10 years ago. Furthermore, diphtheria seroprotection decreased with age ($p<0.001$) and was less attained in COPD and SOT patients compared to DM1 patients ($p<0.01$). Tetanus seroprotection was less reached in women ($p<0.001$) and older age groups ($p<0.001$). For pertussis, women had more often a titer suggestive of a recent infection or vaccination (≥100IU/ml, $p<0.01$). We concluded that except for tetanus, the vast majority of at-risk patients remains susceptible for vaccine preventable diseases such as diphtheria and pertussis.

Furthermore, in **chapter 6** we evaluated the seroprevalence of antibodies against measles, mumps, rubella, diphtheria, tetanus and pertussis in 222 pediatric at-risk patients, aged 2-21 years, with allergies (n=14), congenital heart disease (n=25), diabetes type 1 (n=58), cystic fibrosis (n=9), primary immunodeficiency (n=88) or a history of a SOT (n=28). The seroprevalence of antibodies was 83.3% for measles (≥150mIU/ml), 82.9% for mumps (≥230 titers/ml) and 80.6% of children were protected against rubella (≥10IU/ml). Most patients were protected against tetanus (≥0.1IU/ml) (93.2%), but only 61.3% were protected against diphtheria (≥0.1IU/ml) and 53.2% had antibodies (≥5 IU/ml) against pertussis. SOT patients had the lowest seroprevalence rates for all studied vaccine-preventable diseases except tetanus. Age-appropriate vaccination was associated with a higher seroprevalence, albeit not significant for diphtheria and pertussis. We concluded that a substantial proportion of children with chronic disease remain susceptible to vaccine-preventable diseases. This is partly explained by a relatively low vaccination coverage (73% for DTP to 85% for MMR) and possibly also by the impact of chronic diseases and associated treatment on the immune system. Our findings highlight the importance of close follow-up of the vaccination status in children with chronic diseases.

Part II of this PhD thesis concerned indirect protection provided by vaccination, which is the protection provided to a non-immune person when the people in the community or the close contacts of the persons are immune. The latter includes household members but also healthcare workers (HCWs) who care for vulnerable patients in hospitals or long-term care facilities (LTCFs). In this context, we assessed in the vaccination status and determinants of vaccination in more than 5000 HCWs from 13 hospitals and 14 long-term care facilities (**chapter 7)**. We found a mean 5-year vaccination coverage of about 40% in the hospitals and about 45% in the LTCFs. Overall, up to 90% of HCWs found it important not to infect their patients. However, only 20% of non-vaccinated HCWs considered influenza vaccination a duty to not harm their patients. Up to 40% of unvaccinated staff believed they could get influenza after vaccination and that vaccination weakens their immune system. Also, only about 20% of unvaccinated staff thought to have a high chance of getting influenza. Reasons for unvaccinated staff to get vaccinated in the future are self-protection and protection of family members. Factors that positively influenced vaccination coverage are encouragement by supervisors (OR, hospitals: 7.1, $p<0.001$; nursing homes: 7.5, $p<0.001$) and well-organized vaccination campaigns with on-site vaccination. Factors that negatively affected vaccination

coverage are misconceptions about influenza and its vaccine (OR, range 0.1-0.7, p<0.001 for most misconceptions) and underestimation of the risk of contracting influenza by patients or HCWs (OR of perceived susceptibility, range 2.1-5.1, p<0.001 for most factors). Based on the results of this study and international literature a guidance document for the organization of seasonal influenza campaigns, in which education, communication and easy-accessible vaccination are promoted, was developed. The ready-to-use instruction manual contains a stepwise approach with 24 possible interventions ranging from preparatory work to campaign evaluation. It includes easy-access vaccination, role model involvement, personalised promotional material, education and extensive communication. The manual is available at the website of the Flemish agency for Health and Care (http://www.laatjevaccineren.be/hou-griep-uit-je-team). In **chapter 8,** we evaluated the usefulness of this manual and its impact on vaccination uptake, attitudes towards influenza vaccination and reasons for vaccine acceptance in 11 LTCFs during the vaccination campaign preceding the 2017/2018 influenza season We found higher vaccination uptake rates and decreased perceived barriers towards influenza vaccination in LTCFS that used the instruction manual. In particular, the mean vaccination coverage reported by the LTCFs was 54% (range: 35-72%) in 2016 and 68% (range: 45-81%) in 2017. The coverage increased with 10-30% in LTCFs that implemented at least seven out of 24 possible interventions. Three LTCFs reached or exceeded the target of 80% set by the Flemish government and two approached it with a vaccination coverage of 79%. We also found that HCWs were less likely to expect side effects, to oppose vaccination, to believe that influenza vaccination is used to decrease sick leave or to believe that vaccination is useless due to the many visitors the residents have. The manual was found useful by the organizers of the campaigns. Based on these results, we concluded that the intervention manual supports vaccination uptake and decreases perceived barriers towards influenza vaccination. This is especially true when LTCFs are motivated and willing to invest time and financial resources.

Overall, we concluded that many at-risk patients are inadequately vaccinated and remain susceptible to vaccine-preventable diseases. For this reason, we advocate for a closer follow-up of vaccination status. For SOT patients we emphasize on the importance of pre-transplantation vaccination. This is important given the suboptimal seroconversion after receiving the 9vHPV vaccine and the high susceptibility for other vaccine-preventable diseases. Furthermore, to optimize protection in at-risk patient, advocate vaccinating close contacts of vulnerable patients, especially against vaccine-preventable diseases, which are still endemic such as influenza, pertussis, measles and mumps. Vaccination of close contacts includes vaccination of HCWs. We found that HCWs are sub-optimally vaccinated against influenza, but that vaccination coverage can increase after implementation of a well-organized multi-intervention campaign.

Nederlandstalige samenvatting

Vaccinatie biedt niet enkel directe bescherming aan de gevaccineerde persoon, maar draagt ook bij aan de bescherming van de hele gemeenschap wanneer een groot deel van deze populatie wordt gevaccineerd. Door groepsimmuniteit kunnen ook kwetsbare personen, met name degenen die niet kunnen worden gevaccineerd en degenen die niet reageren op vaccinatie, worden beschermd. Dit doctoraatsproject omvat verschillende studies die verband houden met de directe en indirecte bescherming van risicopatiënten en -individuen.

Deel I van deze doctoraatsthesis betrof directe bescherming na vaccinatie. Ten eerste hebben we gekeken naar de immunogeniciteit of het vermogen van een vaccin om een immuunreactie op te wekken (**hoofdstuk 3**). Meer bepaald bestudeerden we de immunogeniciteit en veiligheid van het 9vHPV-vaccin bij 100 HIV- en 171 vaste orgaantransplantpatiënten in een fase III klinische proef. Dit was de eerste studie die het 9vHPV-vaccin bij deze populaties evalueerde. We stelden vast dat bij alle HIV-patiënten een immuunantwoord voor alle HPV-typen kon opgewekt worden, maar dat dit bij transplantpatiënten varieerde van 46% voor HPV45 tot 72% voor HPV58. In de groep van patiënten met HIV werden significant hogere antistoftiters bereikt bij patiënten met Afrikaanse roots voor alle HPV-typen behalve HPV6 en HPV11. Er was geen duidelijk effect van het aantal CD4+ cellen. Bij transplantpatiënten was de seroconversie lager voor alle onderzochte HPV-typen wanneer de patiënt mycofenolaatmofetil of tacrolimus therapie ondergingen. Dit was echter enkel significant voor mycofenolaatmofetil. Het vaccinatieschema met 3 dosissen van het 9vHPV-vaccin werd in beide patiëntengroepen goed verdragen.

In een tweede project, keken we of de richtlijnen van het aanbevolen vaccinatieprogramma voldoende opgevolgd werden bij risicopatiënten met als doel optimale bescherming te bereiken in deze groep. Er werd een groep pediatrische en volwassen patiënten gescreend op hun vaccinatiestatus en mate van bescherming onder de vorm van circulerende antistoffen tegen enkele vaccineerbare ziekten. In **hoofdstuk 4** beschrijven we de vaccinatiestatus voor influenza, pneumokokken, hepatitis B en difterie, tetanus en kinkhoest en determinanten van vaccinatie bij volwassenen met chronische ziekten. We includeerden patiënten met diabetes mellitus type 1 (n=173) en type 2 (n= 77), chronische nierfalen (n=138), hartfalen (n=200), chronische obstructieve longziekte (COPD) (n=187), HIV (n=201) of orgaantransplantatie (n=255) in een monocentrisch onderzoek. In totaal was 29% van de proefpersonen gevaccineerd tegen difterie-tetanus, 10% tegen kinkhoest, 44% tegen influenza, 32% tegen pneumokokkenziekte en 24% van de HIV-patiënten en 31% van de CKD-patiënten tegen hepatitis B. Leeftijd was geassocieerd met vaccinatie tegen influenza (OR: 2.0, p <0.01) en pneumokokkenziekte (OR: 2.6, p <0.001). Patiënten met COPD, HIV of een transplantorgaan hadden meer kans om gevaccineerd te zijn tegen influenza (OR: 2,8, p <0,001, OR: 1,8, p <0,05; OR: 2,0, p <0,001, respectievelijk) en pneumokokken (OR: 2,9, p <0,001, OR: 25,0, p <0,001; OR: 2,6, p <0,001, respectievelijk) dan patiënten met hartfalen. Reden waarom het vaccin niet werd toegediend waren twijfels over de effectiviteit, noodzaak en bijwerkingen van griepvaccinatie, en niet op de hoogte zijn van de aanbeveling voor pneumokokkenvaccinatie. Onze conclusie is dat er initiatieven nodig zijn om de

vaccinatiestatus van kwetsbare patiënten op te volgen en we pleiten daarom voor systematische vaccinatieregistratie en frequente communicatie over vaccinatie.

Daarnaast rapporteren we in **hoofdstuk 5** over de seroprevalentie van antilichamen tegen difterie, tetanus en pertussis die geanalyseerd werd bij volwassen risicopatiënten met diabetes mellitus type 1 (DM1) (n=172), DM2 (n=77), chronische nierziekte (n=130), chronische obstructieve longziekte (COPD) (n=170), hartfalen (n=77), HIV (n=196) of een geschiedenis van vaste orgaantransplantatie (n=230). Beschermende titers werden bereikt in 29% voor difterie (≥ 0,1 IU/ml), in 83% voor tetanus (≥ 0,1 IU/ml) en seropositieve titers in 22% voor kinkhoest (≥ 5 IU/ml). De beschermingspercentages waren hoger ($p<0,001$) wanneer de patiënt gevaccineerd werd in de laatste 10 jaar. Verder nam de bescherming tegen difterie af met de leeftijd ($p<0,001$) en was deze lager bij COPD- en transplantpatiënten in vergelijking met DM1-patiënten ($p<0,01$). Bescherming tegen tetanus werd minder bereikt bij vrouwen ($p<0,001$) en oudere leeftijdsgroepen ($p<0,001$). Wat pertussis betreft, hadden vrouwen vaker een titer die duidt op een recente infectie of vaccinatie (≥100 IU/ ml, $p<0,01$). We concludeerden dat, behalve voor tetanus, een groot deel van de risicopatiënten vatbaar blijft voor vaccineerbare ziekten zoals difterie en pertussis.

Bovendien beschrijven we in **hoofdstuk 6** over de evaluatie van de seroprevalentie van antilichamen tegen mazelen, bof, rubella, difterie, tetanus en kinkhoest bij 222 pediatrische risicopatiënten van 2-21 jaar oud. Het betrof patiënten met allergieën (n=14), aangeboren hartaandoeningen (n=25), diabetes type 1 (n=58), cystische fibrose (n=9), primaire immunodeficiëntie (n=88) of een voorgeschiedenis van vaste orgaantransplantatie (n = 28). De seroprevalentie van antilichamen was 83,3% voor mazelen (≥150 mIIU/ml), 82,9% voor bof (≥230 titers/ml) en 80,6% van de kinderen was beschermd tegen rubella (≥10 IU/ml). De meeste patiënten waren beschermd tegen tetanus (≥ 0,1 IU/ml) (93,2%), maar slechts 61,3% was beschermd tegen difterie (≥0,1 IU/ml) en 53,2% had antilichamen (≥ 5 IU/ml) tegen pertussis. Transplantpatiënten hadden de laagste seroprevalentie voor alle onderzochte vaccineerbare ziekten behalve tetanus. Seroprevalentie was hoger bij kinderen die correct gevaccineerd waren volgens de aanbevelingen, ook al was dit niet significant voor difterie en kinkhoest. We concluderen uit deze studie dat een aanzienlijk deel van de kinderen met chronische ziekten vatbaar blijft voor vaccineerbare ziekten. Dit wordt enerzijds verklaard door een relatief lage vaccinatiegraad (73% voor difterie-tetanus-pertussis tot 85% voor mazelen-bof-rubella) en anderzijds mogelijk door de impact van chronische ziekten op het immuunsysteem. Deze bevindingen benadrukken het belang van een nauwgezette opvolging van de vaccinatiestatus bij kinderen met chronische ziekten.

Deel II van deze doctoraatsthesis ging over indirecte bescherming door vaccinatie of de bescherming die wordt geboden aan een niet-immuun persoon wanneer het merendeel van de populatie of de dichte contacten van de personen immuun zijn. Onder deze laatste noemer vallen gezinsleden, maar ook gezondheidswerkers die zorgen voor kwetsbare patiënten in ziekenhuizen of zorginstellingen. In **hoofdstuk 7** hebben we de vaccinatiestatus en determinanten van griepvaccinatie bij meer dan 5000 gezondheidswerkers van 13 ziekenhuizen en 14 woonzorgcentra onderzocht. We vonden een vaccinatiegraad (gemiddelde van vijf jaren) van ongeveer 40% in de ziekenhuizen en ongeveer 45% in de

woonzorgcentra. In totaal vond tot 90% van de gezondheidswerkers het belangrijk om hun patiënten niet te besmetten. Slechts 20% van de niet-gevaccineerde gezondheidswerkers beschouwde griepvaccinatie echter als een plicht om geen schade te berokkenen aan patiënten. Tot 40% van de niet-gevaccineerde gezondheidswerkers was van mening dat ze na vaccinatie griep zouden kunnen krijgen en dat vaccinatie hun immuunsysteem verzwakt. Ook dacht slechts ongeveer 20% van het niet-gevaccineerde personeel een grote kans te hebben om griep te krijgen. Redenen voor niet-gevaccineerd personeel om zich in de toekomst toch te laten vaccineren, waren zelfbescherming en bescherming van gezinsleden. Factoren die de vaccinatiegraad positief beïnvloedden, waren aanmoediging door supervisors (OR, ziekenhuizen: 7,1, p <0,001; woonzorgcentra: 7,5, p <0,001) en goed georganiseerde vaccinatiecampagnes. Factoren die de vaccinatiegraad negatief beïnvloedden, waren geloof in mythes over influenza en het influenzavaccin (OR-range: 0,1-0,7, p<0,001 voor de meeste mythes) en onderschatting van vatbaarheid voor influenza voor zowel patiënten als gezondheidswerkers zelf (OR range: 2,1-5,1, p <0,001). Op basis van deze gegevens en internationale literatuur werd vervolgens handleiding met richtlijnen voor de organisatie van griepvaccinatiecampagnes, waarin opleiding, communicatie en toegankelijke vaccinatie centraal staan, ontwikkeld. De handleiding bevat een stapsgewijze aanpak met 24 mogelijke interventies, gaande van voorbereidend werk tot evaluatie van de campagne. Het behandelt aspecten zoals toegankelijke vaccinatie, betrokkenheid van rolmodellen, gepersonaliseerd promotiemateriaal, opleiding en uitgebreide communicatie. De handleiding is beschikbaar op de website van het Vlaams Agentschap voor Zorg en Gezondheid (http://www.laatjevaccineren.be/hou-griep-uit-je-team). In **hoofdstuk 8** hebben we het nut van deze handleiding geëvalueerd in 11 woonzorgcentra tijdens de vaccinatiecampagne voorafgaand aan het griepseizoen 2017/2018. We bekeken het effect op de vaccinatiegraad, de houding ten opzichte van griepvaccinatie en de redenen voor vaccinatie-aanvaarding bij gezondheidswerkers. We vonden verhoogde vaccinatiegraad en verminderde waargenomen barrières voor griepvaccinatie bij gezondheidswerkers uit woonzorgcentra die handleiding gebruikten. De gemiddelde vaccinatiegraad die door de woonzorgcentra werd gerapporteerd was 54% (spreiding: 35-72%) in 2016 en 68% (spreiding: 45-81%) in 2017. De vaccinatiegraad steeg met 10-30% in woonzorgcentra die minimaal zeven van de 24 mogelijke interventies implementeerden. Drie woonzorgcentra bereikten of overschreden zelfs de door de Vlaamse overheid opgestelde doelstelling van 80% en twee woonzorgcentra benaderden deze doelstelling met een vaccinatiedekking van 79%. We vonden ook dat gezondheidswerkers minder snel bijwerkingen verwachten, zich minder tegen vaccinatie verzetten, minder geloven dat griepvaccinatie wordt gebruikt om ziekteverzuim te verminderen of dat vaccinatie nutteloos is vanwege de vele bezoekers die de bewoners hebben. De handleiding werd ook nuttig bevonden door de organisatoren van de campagnes. Op basis van deze resultaten concluderen we dat de handleiding vaccinatie bij gezondheidswerkers ondersteunt en barrières ten opzichte van griepvaccinatie vermindert. Dit geldt vooral wanneer woonzorgcentra gemotiveerd zijn en bereid om tijd en financiële middelen te investeren in de campagne.

De algemene conclusie van deze doctoraatsthesis is dat veel risicopatiënten onvoldoende zijn gevaccineerd en vatbaar blijven voor vaccineerbare ziekten. Om die reden pleiten wij voor een nauwere

opvolging van de vaccinatiestatus van deze patiënten. Voor transplantpatiënten benadrukken we het belang van vaccinatie voor de transplantatie. Dit is belangrijk gezien de suboptimale seroconversie na toediening van het 9vHPV-vaccin en de hoge vatbaarheid voor andere vaccineerbare ziekten. Om de bescherming bij risicopatiënten te optimaliseren, raden we bovendien vaccinatie van de dichte contacten van de kwetsbare patiënten sterk aan. Hierbij leggen we de nadruk op vaccinatie tegen infectieziekten die nog steeds endemisch zijn zoals influenza, pertussis, mazelen en bof. Vaccinatie van dichte contacten omvat ook vaccinatie van gezondheidswerkers. We vonden dat gezondheidswerkers niet optimaal zijn ingeënt tegen influenza, maar dat de vaccinatiegraad kan toenemen na uitvoering van een goed georganiseerde campagne met meerdere interventies.

Scientific acknowledgements and personal contribution

Chapter 3: Immunogenicity and safety of the nine-valent HPV vaccine in solid organ transplant and HIV patients

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Obtaining funding: Lise Boey and Corinne Vandermeulen

Protocol and study design: draft Lise Boey and Corinne Vandermeulen, revision all other authors

Study visits: During the period of October 2018 until April 2019, Lise Boey received help from Ans Curinckx, who assisted in the study in the context of her master thesis in Biomedical Sciences.

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Manuscript draft: Lise Boey

Extensive revision of manuscript: all authors

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Chapter 4: Vaccination coverage of recommended vaccines and determinants of vaccination in at-risk groups

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Obtaining funding: Corinne Vandermeulen

Protocol and study design: Corinne Vandermeulen

Data collection: Lise Boey (diabetes type 2, data from general practioners for chronic kidney disease, guidance of master thesis students Eline Bosmans and Lisa Smans), Eline Bosmans (SOT patients), Liane Braz Ferreira (patients with cardiovascular disease), Nathalie Heyvaert (COPD patients), Melissa Nelen (diabetes type 1 and chronic kidney disease), Lisa Smans (SOT patients), Hanne Tuerlinckx (HIV patients)

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Statistical analysis: Lise Boey and Mathieu Roelants

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Extensive revision of manuscript: all authors

Chapter 5: Seroprevalence of antibodies against diphtheria, tetanus and pertussis in adult at-risk patients

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Analysis of blood samples: Isabelle Desombere and her team from sciensano

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Extensive revision of manuscript: all authors

Chapter 6: Seroprevalence of antibodies against vaccine-preventable diseases in children with chronic diseases

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Statistical analysis: Lise Boey and Mathieu Roelants

Manuscript draft: Lise Boey

Extensive revision of manuscript: all authors

Chapter 7: Attitudes, beliefs, determinants and organizational barriers behind the low seasonal influenza vaccination uptake in healthcare workers – A cross-sectional survey

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Obtaining funding: Corinne Vandermeulen

Protocol and study design: Corinne Vandermeulen, Mathieu Roelants, Lode Godderis, Antoon Deschryver and Karel Hoppenbrouwers

Data collection*: Charlotte Bral*

Statistical analysis: Mathieu Roelants, Corinne Vandermeulen and Charlotte Bral

Manuscript draft: Lise Boey

Extensive revision of manuscript: all authors

Chapter 8: Increased vaccine uptake and less perceived barriers towards vaccination in long-term care facilities that use multi-intervention manual for influenza campaigns

Authors: Lise Boey, Mathieu Roelants, Corinne Vandermeulen

Obtaining funding: Corinne Vandermeulen

Protocol and study design: Lise Boey and Corrine Vandermeulen

Data collection*: Lise Boey*

Statistical analysis: Lise Boey, Mathieu Roelants, Corinne Vandermeulen

Manuscript draft: Lise Boey

Extensive revision of manuscript: all authors

Conflict of interest statement

CV was principal investigator for vaccine clinical trials from GSK, MSD, AdImmune and Pfizer for which the university received grants. CV received no personal grants. RV is a senior clinical research fellow of the Research Foundation Flanders (FWO), but received no specific funding for the current study. All the other authors have nothing to disclose.

Professional career

Lise Boey obtained a master degree in Biomedical Sciences in 2016 in Leuven, Belgium. Thereafter, she started a PhD on vaccination in at-risk populations at the Leuven University Vaccinology Center (LUVAC) under the supervision of prof. dr. Corinne Vandermeulen. Lise Boey has performed as well qualitative as quantitative research. Particularly, she has surveyed several patient populations on vaccination coverage, determinants of vaccination, and reasons for non-vaccination. In addition, she assessed seroprevalence of antibodies against infectious diseases in these populations. Lise Boey also performed policy-guided research. She developed a ready-to-use manual to facilitate implementation of interventions that are known to increase vaccination coverage in healthcare institutions. She evaluated the use of this manual and its effect on vaccination coverage and attitudes of healthcare workers towards influenza vaccination. Next to this, Lise Boey has also performed a clinical trial on the safety and immunogenicity of a nine-valent human papilloma virus (HPV) vaccine in HIV and transplant patients. From 2016-2020 she has supervised master students from biomedical sciences, pharmacy or nursing who made their master thesis in vaccinology. In 2017, she taught a class on the history of vaccination to a group of PhD students from all over Europe during a summer school in Leuven. In 2017-2018, in the framework of a new Honours Program on Transdisciplinary insights Lise Boey teamed up with her PhD supervisor and an international group of students from different faculties of the university to analyze the currently very present complex problem of vaccine hesitancy. She enjoys and values working in a multidisciplinary research field as she believes that complex problems can only be defined and understood by taking into account viewpoints of several people with diverse backgrounds.

List of publications

International peer-reviewed journals

1. Boey L, Bral C, Roelants M, De Schryver A, Godderis L, Hoppenbrouwers K, et al. Attitudes, believes, determinants and organisational barriers behind the low seasonal influenza vaccination uptake in healthcare workers – A cross-sectional survey. Vaccine 2018;36:3351–8. https://doi.org/10.1016/J.VACCINE.2018.04.044.

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Papers to be submitted

1. Boey L, Bosmans E, Braz Ferreira L, Heyvaert N, Nelen M, Smans L, Tuerlinckx H, Roelants M, Claes K, Derdelinckx I, Janssens W, Mathieu C, Van Cleemput J, Vos R, Desombere I, Vandermeulen C. Seroprevalence of antibodies against diphtheria, tetanus and pertussis in adult at-risk patients.

1. Boey L, Curinckx A, Roelants M, Derdelinckx I, Van Wijngaerden E, De Munter P, Vos R, Kuypers D, Van Cleemput J, Vandermeulen C. Immunogenicity and safety of the nine-valent HPV vaccine in solid organ transplant and HIV patients.

2. Boey L, Spiessens T, Loix S, Thijssen K, Cools S, Roelants M, Bullens D, Casteels K, De Boeck K, Gewillig M, Van Geet C, Knops N, Meyts I, Hutse V, Desombere D, Van Gucht S, Vandermeulen C. Seroprevalence of antibodies against vaccine-preventable diseases in children with chronic diseases.

Presentations at international conferences

Oral presentations

1. Vaccination coverage and determinants of under-immunization in immunocompromised children. 19th EUSUHM congress youth health care in Europe, Leuven (Belgium), September 2017.

2. Vaccine coverage and seroprotection against vaccine-preventable diseases in children with chronic diseases. European Society for Paediatric Infectious Diseases (ESPID), Malmö (Sweden), May-June 2018.

3. Safety of the nine-valent HPV vaccine (gardasil®9) in transplant and HIV patients. EUROGIN 2019, international multidisciplinary HPV congress, Monaco, December 2019.

Poster presentations

1. To have or not to have: What motivates healthcare workers in having the seasonal influenza vaccine? International society for vaccines (ISV) congress, Paris (France), October 2017.

2. What motivates healthcare workers in getting vaccinated against seasonal influenza? Seminar on Diagnosis and surveillance of infectious disease, Brussels (Belgium), May 2017.

3. Vaccine coverage and seroprotection against vaccine-preventable diseases in immunocomprised children. European Society for Paediatric Infectious Diseases (ESPID), Malmö (Sweden), May-June 2018.

4. Can Healthcare Workers Be Convinced to Take Up the Seasonal Influenza Vaccine Without Making It Mandatory? The annual conference on vaccinology research (ACVR), Baltimore (USA), April 2019?

5. Development and implementation of an instruction manual for the organization of performant and successful vaccination campaigns in order to increase influenza vaccination uptake in healthcare workers. Seminar on “Diagnosis and surveillance of infectious disease, Brussels (Belgium), May 2019.

6. Seroprevalence of diphtheria, tetanus and pertussis in patients with chronic diseases. International society for vaccines (ISV) congress, Ghent (Belgium), October 2019.